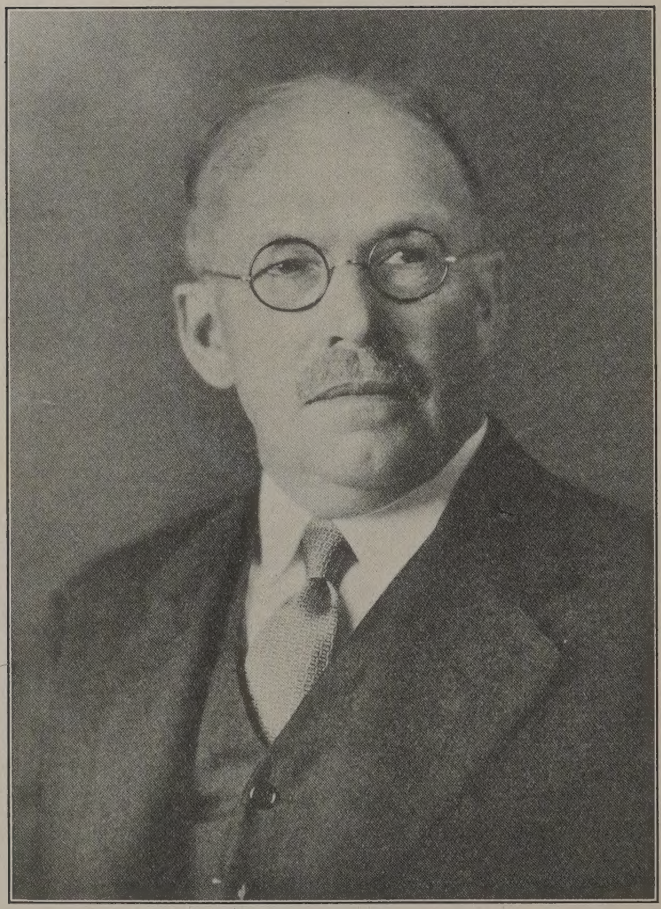


Ernest Edward Tyzzer

The Journal of Parasitology

Volume 21 FEBRUARY, 1935 Number 1

VIEWPOINTS AND ORIENTATION IN PARASITOLOGY[1]

ERNEST EDWARD TYZZER, M.D.

Department of Comparative Pathology, Medical School and School of Public Health, Harvard University

It has occurred to me that a somewhat general survey of the field of Parasitology from a more or less detached standpoint might be helpful, especially to the young investigator. I am not attempting, therefore, a learned discourse, but rather an informal review of the position of Parasitology in relation to other subjects, together with its techniques, opportunities and prospects. Many factors are concerned in determining the nature of investigation, such as material, special talent, training, introduction to certain types of problem through association with those previously engaged upon them, and the peculiar appeal of certain subjects. Whatever such factors may be, it is probably desirable in the course of one's prospecting to pause and survey the landscape before settling down to work on any given claim.

We may first consider what might be called the social standing of Parasitology. In the minds of many, the term "Parasitology" signifies something loathsome and having to do with worms—hardly a proper subject for conversation. This is illustrated by the reaction of a well known educator in regard to a faculty appointment.—"Dr. John Smith, instructor in Helminthology, proposed for promotion to Assistant Professor. I should say that anyone who is interested in Helminthology deserves promotion." Even many medical men inured to constant contact with disease with all its unattractive features, are prone to avoid any reference to worms. Hence, when some one high in the social register is clearly demonstrated to have pin worms, it is a downright calamity, a situation that justifies desperate measures, an appendectomy or some other major operation. Also there appears to be a well established tradition in the profession that the organisms dealt with in Parasitology courses are of slight consequence. Thus an intern who has not forgotten quite all his instruction, on finding *Diphyllobothrium* eggs in a stool is for a time subjected to more or less ridicule by the staff members of his service. A mode of diagnosing and removing tapeworms which seems

[1] Address of the Retiring President, American Society of Parasitologists, December 28, 1934, Pittsburgh.

to be quite unique is by incision of the gall bladder. The proglottids may then be withdrawn yard by yard through the incision and if fortune favors, the scolex also. When a faculty member discovers tapeworm segments in his discharges, it may be quite a difficult matter to convince him that he is not harboring a brand new species of parasite. There has been, however, notable progress: kala azar spleens are no longer removed prior to diagnosis, and it is quite widely recognized that schistosomiasis may be diagnosed without excision of bladder tissue. The parasites themselves are contributing much towards the enlightenment of the medical profession, for they are no respecters of persons and when physicians or members of their families contract amoebic dysentery or trichinosis, the conclusion is reached that parasites after all are worth knowing something about.

Perhaps we are ourselves to blame for the failure of due recognition of our subject. Its history is one of negelcted opportunities. The beauty of many parasites could well be made a basis of appeal to the aesthetic sensibilities rather than a cause for disgust. Can any one imagine more perfect grace and symmetry than is displayed in the sinuous curves of a youthful nematode, or anything more delicately beautiful than the pearly vitellaria of certain monostomes? Already scorpions, crayfish, and the like are coming to be represented on the outer walls of biological laboratories where they serve to inspire the student and the budding scientist. Consider the possibility of the use of festoons of proglottids, mosaics of Chloromyxum spores, and trypanosomes, while entomology could very well furnish a goodly number of appropriate forms for use in architecture or interior decoration. Surely the ancients would not have failed to utilize such forms, as well as those revealed by the microscope, and doubtless fame and fortune awaits the architect who utilizes this wealth of material. In the meantime, more often than not, the student gazes upon austere external contours, and inner walls as blank as those of any manufacturing plant.

Again Parasitology has not received its share of publicity. I am referring to the arousal of public interest in the subject, not of course to those cases of exhibitionism or outright advertising which now and again attract attention. The great interest in hookworm disease aroused by Dr. Stiles is an example of what may be accomplished through justifiable publicity, but in general we do very little in acquainting the public with our home problems, perhaps by reason of fear that anything attempted might be misconstrued. The Rockefeller Foundation has done much in the way of education concerning certain regional parasitic diseases, but with a purely utilitarian objective rather than on the basis of intellectual interest. The public has considerable information about dinosaurs and their eggs and it is likely that it would be quite as much interested in the

parasites that bit the dinosaurs. The life histories of many well known parasites and insect pests, such as fleas, mosquitoes, black flies, and midges, would be of great general interest were they properly presented, and there is no reason why the study of the distribution and the life histories of some of these forms should not be undertaken as hobbies. The identification of mosquitoes and local surveys bearing on the regional and seasonal distribution of species could very well be engaged in by amateurs and much might be learned of the habits of certain species and the local sources of mosquito nuisances. Would not the statement that,—"From the number of Culex larvae now developing in our pools, the prospects for this season's crop of mosquitoes are most promising,"—compare favorably with much of our local news?

Having viewed our subject somewhat introspectively, let us now turn to the qualifications of one entering upon investigation in Parasitology. Neither a formal course of training nor a degree is required. It is customary to expect of the beginner that he demonstrate ability for independent and original work and that he be able to satisfy the criticism of others already in the field. There is probably greater freedom here than in almost any field of science, the problem, the material and the method are largely matters of individual choice. While the description of species and taxonomic studies are of primary importance, the physiology of parasites furnishes problems of great interest. A great deal of study has been devoted to the effects of parasitism in reference to disease, but much still remains to be done, and the broader ecological aspects of the subject have been largely neglected. I have long felt that the parasitologist and the biologist as well would profit by knowing something of general pathology. This means preparation in normal histology, and physiology, in addition to the preliminary subjects of biology, chemistry and physics. Some training and acquaintance with bacteriology and immunology are also essential for certain problems.

As to methods, there is much to be said in favor of combining methods and in utilizing the results of one method to check those obtained by another. Doubtless, direct observation, based as it is on visual evidence carries greatest conviction in almost any type of investigation. Dobell has aptly remarked "Yet there is sometimes more virtue in one accurate observation than in ninety-nine inconclusive experiments." The numerous aids to biological observation are so well known that it is not necessary to enumerate them.

The experimental method, although widely adopted and extremely useful, may fall short of the mark when employed without support of other evidence. The results obtained in animal experimentation may be crude and unilluminating if not supplemented by histological study, and results are much more significant when supported by a study of the

processes which lead up to them. In animal experimentation, we frequently employ a very artificial set-up in order to eliminate as far as possible all variables except those with which we wish to deal. This is not only proper but is also more or less essential to progress, and by employing such measures we may finally be able to prophesy the outcome of the interaction of a given combination of influences,—a very satisfactory state to reach. The limitations of the laboratory experiment should not be lost sight of, however. I was once told of a British helminthologist who, after being appointed on a Commission to investigate certain agricultural problems, was more or less pestered with inquiries as to what he could do for the farmer. Finally in desperation, he replied that the best advice he could offer the farmers was for them not to believe in anything that came from the laboratory. While an extreme statement, it nevertheless gives emphasis to the point in question, that is, the danger of making generalizations or applications on data either too limited or unrelated to the problem. We may, for example, require for an experiment a homogeneous stock of animals, and employ an inbred stock derived from a long series of brother-sister matings, and yet in nature the brother-sister matings may almost never occur. Again, many experiments hang on a single event, such as an inoculation, and at most they involve relatively brief periods of exposure of an animal to given conditions. In nature's experiments, such exposure may continue through the entire life of an animal, while species may be exposed for untold millenia. We may fail to transfer the parasite of human malaria to an ape, but we do not know what the result would be if we could continue to inoculate apes for several thousands of years.

A type of experiment which has long been emphasized by Theobald Smith is the experiment of nature that comes "ready-made" so to speak, but in which the conditions of the experiment are known. Such a spontaneous experiment, the "accident experiment" of Bacon, often yields information not to be obtained from any planned program. Thus experimental infection in the case of tuberculosis furnished no adequate explanation for human phthisis until supplemented by a study of the natural history of the disease in the human being extending over many decades. It is now pretty well demonstrated that the adult type of pulmonary tuberculosis results from the reinfection of individuals rendered hypersensitive by infection occurring in childhood. Thus by the comparative observational method of treating the data furnished by nature's experiments, we have not only a useful check on the applicability of results obtained by laboratory experiments, but also a means of determining how these fit into the picture as a whole. To quote Dr. Smith: "The comparative method is frequently in position to restrain generalizations deduced from experimental procedures and to keep the experi-

menter from steering away from the goal, which is an understanding of the totality."

Finally, we have the mathematical method in biological work. In contrast to the importance of this discipline in such sciences as physics, chemistry and astronomy, its usefulness as a tool in biological work is rather difficult to evaluate. It has found application in optics, especially in the building of microscopes and other useful instruments, but in the purely statistical field, its contributions are not especially impressive up to the present time. Perhaps the greatest contribution of the statistical method is found in genetics, and even here the fundamental principles became apparent without any very advanced mathematical procedures. I have a suspicion that there are those who think that the biological sciences do not make the grade, so to speak, unless they are placed on a mathematical basis. We find a distinction stressed between the so-called precise sciences and those that are not. To this distinction, I must take exception, for biological observations or events furnish data in many respects as exact as may be found in any other field. While views as to the nature of force and matter are constantly changing, births and deaths constitute universally accepted facts. In biological work the nature of the data, which in themselves may be of the greatest exactitude, is such that they are not amenable to mathematical treatment owing to the impossibility of controlling all the variables inherent even in the simplest experimental set-up. In physical or chemical investigations, on the other hand, the variables are known and few in number and can usually be accurately controlled, or where control is not possible the effect of the uncontrolled variable is sufficiently well known to enable one to make the necessary correction.

The reliability of data is quite as important as the manner in which they are marshalled and the precision of a method furnishes no guarantee of its appropriateness. It would appear that for some reason or other, the satisfaction derived from the mathematical method tends to supplant any curiosity as to underlying causes. In fact it seems that there is much in common between liquor and mathematics. While there are certain hard-headed individuals who are apparently able to carry considerable amounts of one or the other, it may be well for the biologist to consider the effects of the adoption of mathematics as a technique very carefully before embarking on its habitual use. It is well known that mathematicians are notoriously lacking in biological sense, some addicts having no interest whatever in reality, and it would also appear that the biologist who takes up the habit becomes so enthralled with the exquisite beauty of the technique that so long as he is able to fit a curve, he disregards relevant facts and fails to utilize controls quite obvious to the novice. While the method implies accuracy and precision, it is perhaps oftener than

not accompanied by sloppiness of technique and looseness of thought. Suppose we turn to the enumeration of the corpuscles of the blood, and it is not to be denied that blood counts constitute a very important aid in the study of disease, but even if we compute probable error, it constitutes, as usually carried out, at best only a rough and ready procedure. I once undertook with a well known physicist the calibration of a series of blood-counting pipettes manufactured by one of the most reliable of firms of instrument makers, by weighing the contents on a chemical balance. None of the pipettes were found even approximately correct, the error in some being as much as 12 per cent. Considerable variation in the cell content of different sample drops from the same pipette is encountered. Furthermore, the cell content will vary with the region of the body from which the blood is taken and the manner of its withdrawal. Again let us suppose we are counting coccidium oöcysts in animal feces. In order to sample the material, it is necessary to dilute it, and if we have to deal with a considerable bulk of such material, the dilution may be such that a single oöcyst in an entire microscopic preparation may yield us a count as high as 20,000 for the total sample, while the failure to find an oöcyst merely serves as a rough indication that oöcysts are not present in excess of the number in question. In contrast, we have the biological procedure of feeding such material to uninfected animals, by which we may obtain infection even with a single oöcyst. Thus while the quantitative method here furnishes a very rough approximation and no information as the presence of small numbers, the biological procedure serves as a very delicate test. I am not offering these illustrations as proof of my contention, but merely to point out that there are pitfalls in the employment of the mathematical technique. Quantitative studies are, of course, of great importance in biological work, but it seems that the elaborate treatment of data in the first place seldom proves anything that isn't quite obvious without it, and in the second place subsequently leads to the neglect of acquaintance with material, in other words with reality. After all it is the latter that we are primarily interested in in Parasitology.

The importance in all scientific effort, of the impelling forces of curiosity, imagination and enthusiasm is quite obvious, but it is perhaps well also to consider the objective. Is such effort to be wholly disinterested and directed to the discovery of truth for its own sake, or is it to find out that which is useful? This is too great a question to be discussed at length at this time, but some allusion to it seems called for by the times in which we are living. We may say that the true savant has no utilitarian interests and that he who undertakes practical problems does not qualify as a savant. As in most human undertakings, the motivation in scientific work is apt to be more or less complex or obscure. Some of

our greatest figures in the medical sciences have deliberately chosen practical problems, but no one denies the importance of their contributions. Doubtless the primary object is often forgotten with the revelation of great principles. While training, circumstances and expediency will of necessity influence the career of the investigator, his course will depend largely on his choice of problems, his interests and his realization of the significance of phenomena. One will require tangible results for his effort, another will look more deeply and doubtless all gradations may be found between the two extremes. There is to my mind no question as to the relative quality of the two types of effort and at the present day in which there is a pronounced swing toward the utilitarian in science, the major importance of the contributions of the savant should not be forgotten.

There appears to be a growing attitude of impatience toward scientific activity which is not concerned in what are considered to be the major problems of the day. Doubtless the problems of government and social economy are more important and more difficult than those of scientists of the type alluded to as "devoting their lives to the study of the hind leg of a flea." It must be granted, however, that those engaged in the biological sciences have acquired an attitude of mind, and a methodology which enable them to cope with their problems with a high degree of success, an equipment which is notably lacking in the social sciences. Even though certain subjects appear to have no direct bearing on the pressing problems of the day, it hardly seems desirable that activity should cease in them. On the basis of the value of scientific thought in general, continuity in established fields must be provided for, even though certain realignments must inevitably occur. As pointed out by Mr. Lowell, the university must go on under all circumstances, whether of war, of overthrow of governments, or of any major disturbance.

Turning now to the position of Parasitology with reference to other subjects, one often hears of branches of science, but science is not like a tree. My conception of science as a whole is that it is a living, growing, three-dimensional web or reticulum (perhaps the physicist may be able to improve upon this), in which there are centers of activity differing in kind and degree, but all bound together into a single whole. While marginal branches may appear, the most outstanding character in the organization of science is its free anastomosis, with new possibilities opening up as new centers appear or as activity shifts from one field to another, the whole texture of science being bound up with human experience. Thus, reference to "marginal contacts" of a subject fails to express its relationship, for almost every subject is intimately bound up with other related subjects. In the course of its evolution, it will be noted that new types of activity appear in certain portions of the web,—a science comes

into being. Such activities may increase rapidly for a time, later gradually abate and finally the field may become, if not moribund, at least quiescent. Also new centers may arise within larger fields of activity and increase to such an extent as to acquire a major importance. Thus sciences are born, pass through periods of exuberant youth, maturity and eventually reach a state of senescence, one in which activity is reduced to a minimum. For example, Bacteriology arose within the field of Parasitology, grew rapidly, became linked up with Pathology, Medicine, Public Health, Agriculture, etc., and then within it in turn Immunology arose and played for a time a major rôle. Later we see the activity in Bacteriology shift to the filterable viruses or to problems of disease transmission. A discovery may not only initiate activity in the field in which it is made, but also may at once stimulate activity in other fields, often in distant as well as closely related ones. It seems that the length of time over which notable activity is extended bears a definite relation to at least two factors, first the intensity with which the field is worked and second the limitation or narrowness of the subject.

The boundaries set for most fields of science are arbitrary and more or less a matter of convenience, but there are influences which tend to limit or even contract the field of any given subject. The scientific journal is one of the most important of these narrowing influences. We may point to the necessity of specialization and the convenience of easy access of literature, but on the other hand there is in the policy of many journals a definite tendency towards regimentation in regard not only to the nature of the material submitted but also to the manner of its presentation. Some journals may accept only brief papers and as a result the literature becomes flooded with articles based on isolated experiments, too few to be conclusive and with what amounts to little more than isolated observations. On the other hand it may be found difficult to publish the well rounded treatise based on long acquaintance and ripe investigation. Apparently there are many individuals who would acquire standing on the basis of the number of titles which they have to their credit, and essentially the same paper is published in a number of journals so that in extreme cases we have what amounts to downright advertising. A form of bootlegging makes its appearance—papers are published based on the work of others, to which reference is carefully avoided, and offering no notable contribution of their own. Such activities in publication obviously serve no worthy purpose and should not be encouraged. Some journals may accept only papers in a very limited field and while all the changes are being rung on a subject, the journal furnishes only dull reading. The fact is lost sight of that the interest of a journal is apt to be correlated with the breadth of the field covered and with variety in its papers. With the policy of liberalism and breadth

of view, a journal fulfills a much more important function than when it caters to narrow specialism. Whether the responsibility of a journal's policy rests with the editor or with the society sponsoring it, the effect is very much the same.

The field of Parasitology is a very broad one, so broad that only certain portions of it have been adequately explored. Some subjects have been intensively worked over and it is possible that the greatest activity in these has passed. There appear to be, however, almost unlimited possibilities, if activity is extended in the proper directions, and new approaches and new applications are utilized. Some problems are difficult, perhaps insoluble, but nevertheless from their intrinsic interest require attention, as for example the evolution of parasites and their influence on the evolution and survival of other forms. Thus did the broad tapeworm first parasitize a fish-eating animal, such as the otter, and subsequently get transplanted to man, or did it start in the latter due to the adoption of the lake-dwelling type of abode. The study of parasitism in connection with the origin and migration of higher species, in which Metcalf has done pioneer work, the distribution of parasites in human races as introduced by Darling, are matters of great general interest. In connection with the text-book statements of the introduction of the broad tapeworm in the western hemisphere, I have often wondered what was the nature of the tapeworm mentioned as occurring frequently in the Tinneh Indians of the Canadian northwest a matter of sixty years ago. Parasitism should be taken into account in Physical Anthropology, in Paleontology, especially with reference to species that have become recently extinct, in Archaeology, Ethnology, and even the social sciences. The increasing density of human and domestic-animal populations and the introduction of new pests are constantly giving rise to new problems. Thus while Parasitology may lack the intensity of effort and accompanying interest characteristic of narrower subjects, it may be considered as in the prime of life and gives promise of considerable longevity.

Having introduced certain controversial subjects, I realize that I have not been quite fair to my hearers, since I understand that there is no opportunity on this occasion for general discussion. However agreement on all the subjects discussed is neither expected nor indeed for the present desirable, but I shall be satisfied if I have brought realization that there are many approaches, interesting problems, and promising prospects in our field of endeavor.

CULTIVATION OF *TROGLODYTELLA ABRASSARTI*, A PARASITIC CILIATE OF THE CHIMPANZEE[1]

W. W. Swezey

INTRODUCTION

The only previous report of the cultivation of *Troglodytella abrassarti* is by Nelson (1932) who was able to keep the organisms in culture for seven days. He used the medium of Tanabe and Chiba (1928) for the cultivation of *Endamoeba histolytica*. He was successfully using this medium, at that time, for the cultivation of *Balantidium coli*, but the *Troglodytella* were much more difficult to cultivate. Nelson made no counts to determine whether multiplication actually was taking place. He saw dividing forms in his culture but dividing forms occur in fresh fecal material. Since the writer has been unable to demonstrate multiplication in the medium used by Nelson, the actual cultivation of the ciliate by him seems doubtful.

In order to do extensive experimental work upon this genus it is necessary to cultivate the organisms. The medium used by Nelson (1932) will keep the organisms alive, but will not carry a strain over a long period of time.

MATERIAL AND METHODS

The same medium and conditions used by Nelson (1932) were first employed but no multiplication occurred.

An extract of chimpanzee fecal material was tried after many attempted modifications of various other media had failed. A medium similar to this was being used successfully in this laboratory for the cultivation of other intestinal protozoa. It is prepared by extracting a given amount of fecal material with four times as much Ringer's fluid, by volume. This is allowed to stand for twenty-four hours, to permit thorough extraction, and is then strained through cheese-cloth to remove the large particles. After this it is centrifuged and the supernatent fluid filtered through a Büchner Funnel using Whatman's No. 4 filter paper and infusorial earth. The filtrate is kept in the ice-box and used as needed. Rice starch (7 or 8 milligrams) is added to each culture tube.

In order to prove that multiplication occurred in the culture it became necessary to devise some method of counting the individuals in a single

[1] From the Johns Hopkins University, School of Hygiene and Public Health. This work was aided by a grant from the Committee on Scientific Research of the American Medical Association. The writer wishes to express his indebtedness to Drs. Hegner and Andrews for their suggestions and criticisms and to Drs. Van Volkenburg and Long and the Committee on Common Cold Research from whose chimpanzees the material for this work was obtained.

tube. A pipette similar to that used in the Stoll dilution egg count method for the determination of the number of helminth eggs in a fecal sample was used. This pipette is graduated at 0.075 cc. and 0.15 cc. and is the same as is used in the new counting method for ciliate protozoa (Atchley and Swezey, 1933). The material in the culture tube was thoroughly stirred and a 0.075 cc. sample was removed and counted under a 22 x 30 mm. coverslip. In order to kill and stain the organisms a drop of dilute iodine solution was added to the original 0.075 cc. drop of culture material and the organisms were counted under a 22 x 40 mm. coverslip.

When the tubes were to be counted to determine multiplication, all of the culture tubes were inoculated with a given amount of material from a common suspension of organisms, which had previously been counted. A later count of the culture tubes determines whether or not multiplication has occurred.

The probable, as well as the possible, length of life of the organisms in fecal material was determined by subjecting different portions of a fecal sample to different temperatures. In order to keep the stools from becoming too desiccated at the higher temperatures, various diluting fluids, including tap water, Ringer's, Lock's and normal saline were used. Approximately five cc. of one of these fluids was added to each portion of the stool.

ORIGINAL OBSERVATIONS

Length of Life in Various Media

The length of life of *Troglodytella abrassarti* in the different media tried varied considerably, but in all cases the one containing extract of chimpanzee fecal material prove the best, for the organisms not only multiplied more readily in this medium, but lived for longer periods of time without subculturing. The results of these experiments are presented in Table 1. The tubes were incubated at 37.5 ° C. and examined every day at the same time.

TABLE 1.—*Length of Life in Culture Media, with no Subcultures*

Medium	Number of tubes	Days												
		1	2	3	4	5	6	7	8	9	10	11	12	13
Cleveland's	8	8	8	5	3	3	1							
Schumaker's	8	8	6	3	3	1	1							
Tanabe and Chiba's	18	18	18	16	15	7	7	2	1					
Alfalfa Infusion	11	11	5	1	1									
Chimpanzee Fecal Extract	43	43	40	35	34	29	21	19	19	15	15	13	1	0
St. John's	6	6	4	1	1	1								
Ringer's with no serum	8	3	1											
Ringer's with serum and no charcoal	11	9	9	6	3	3	1							
Ringer's with serum and charcoal	4	4	1	1	1									

This series of experiments seems to indicate that *Troglodytella abrassarti* will live for a longer period of time in an extract of chimpanzee fecal material without subculturing than they will in any of the other culture media tried. However, in all of the media the length of life varied according to the number of organisms inoculated; when many organisms are added the time is increased, but the fecal extract proved the most satisfactory in every case.

The addition of a small amount of rice starch (7 or 8 milligrams) to the cultures each day materially lengthened the life of the organisms. They lived in the same culture tubes for more than four weeks and individual tubes of the series ran for periods up to 36 days.

Cultivation in Chimpanzee Fecal Extract

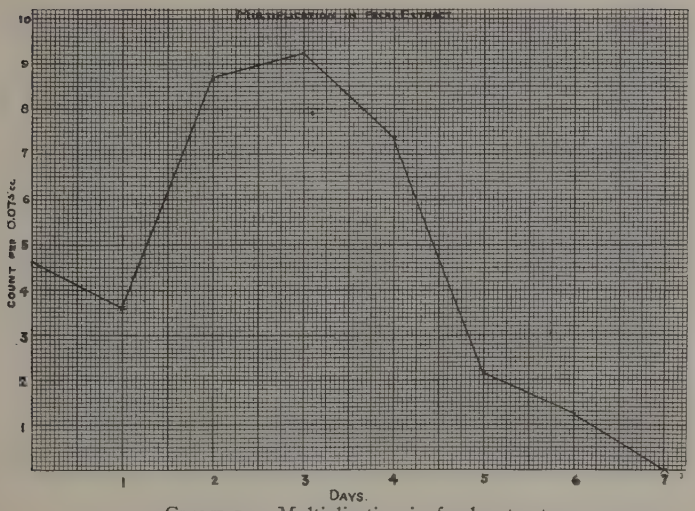
Attempts were made, without counts, to cultivate the organisms in the medium used by Tanabe and Chiba (1928) for the cultivation of *Endamoeba histolytica,* and in other media, but were unsuccessful since the strain, in spite of repeated subcultures, always died out within a few days.

When the extract of chimpanzee fecal material was used, one strain, started in 6 tubes, continued for four weeks, at which time thirty-two tubes were going well, each one apparently containing approximately the same number of organisms as did the original 6 tubes. An increase in the temperature to 41° C., at this time, killed all of the organisms.

A series of thirty-six tubes were inoculated with 0.5 cc. from a common suspension of 680 organisms per cc. and three different tubes were counted each day. In this way no tube was counted two days in succession, so that the possibility of injury by repeated stirring was eliminated. This experiment was repeated twice and the growth curve for these parasites in an extract of chimpanzee feces was plotted in Graph 1. A more thorough study of the culture materials and technique, and the addition of rice starch daily will probably modify this curve.

The results of these counts showed that after the initial drop, due to the killing of some of the organisms in transferring, there was a rapid increase in the number of ciliates up to the high peak on the third day. After that there was a gradual decrease in the number of the organisms until on the seventh day all of those in the tubes were dead. Each point on the graph is the result of an average of a double count on nine tubes. The control tubes, in Schumaker's medium, all died within four days. They showed no appreciable multiplication.

Multiplication of *Troglodytella abrassarti* undoubtedly occurred in these tubes and is the first time this has been demonstrated by actual counting.



GRAPH 1. Multiplication in fecal extract.

Attempted Cultivation in Various Other Media

Attempts were made to cultivate the organisms in media other than fecal extract and Tanabe and Chiba's. The most successful of these was that used by Schumaker (1931) for the cultivation of *Balantidium coli*. This medium does not contain an agar base. Many of the organisms were observed to bore into the agar, when it was used as a base, and thus were lost when attempts to subculture were made. A 10 per cent concentration of serum was found to be optimum for these ciliates for they remained alive and active up to and including the eighth day. No difference between pig, human or dog serum was noted. In 20 per cent serum the organisms died within a few hours, while in 15 per cent and 5 per cent serum they lived for several days. With no serum, two days was the longest that they remained viable. No multiplication apparently occurred in this medium, for repeated subcultures also died out at approximately the same time as the original tubes.

Other media tried, unsuccessfully, have been Cleveland's, with various modifications, alfalfa infusion, St. John's *Endamoeba histolytica* medium, etc. The only really successful medium that has been tried up to this time is the extract, in Ringer's solution, of chimpanzee fecal material.

Length of Life in the Stool at Different Temperatures

To determine the length of life of *Troglodytella abrassarti* in fecal material several experiments, at different temperatures and with various

diluents, were performed. Examinations were made at half-hour intervals to determine the point at which death occurred.

The organisms lived longer when tap water was used as a moistening agent than when Ringer's solution, Locke's solution or normal saline were added to the feces. The reason for this is unknown but the results are given in Table 2. One probable explanation is that the salt concentrations become too high for the organisms due to evaporation of the fluid. It was found, when modifying media for cultivation, that these ciliates cannot live in a high salt concentration.

At room temperature, with tap water as the diluent, the organisms in the 8 stools studied lived for 4½ hours in all and for a maximum period of 7½ hours in 2. During this experiment, all stages of degeneration in the organisms were seen. Many of these corresponded exactly to the description given by Reichenow (1920) to forms which he considered to have been killed and partially digested by the host. While some of the forms he describes may have been partially digested, others obviously were forms in the process of degeneration due to having been in the fecal material too long.

Parts of the stools used in the above experiments were subjected to further temperature studies. At a temperature of 30° C. tap water being used for moistening, the length of life of these ciliates was 28 hours in 2 of the 8 stools, and at least 22 hours in 4 others.

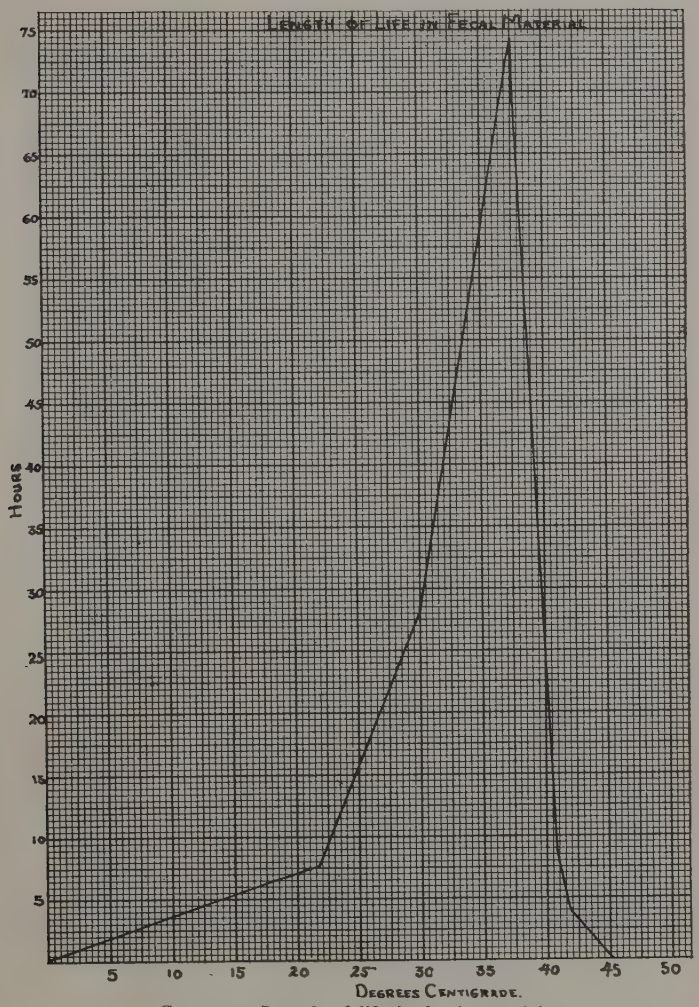
At body temperature (37.5° C.) the life of the infusorians was prolonged to 74 hours when tap water was used, but with Ringer's solution, Locke's solution, or normal saline the organisms died in 50 to 54 hours. Table 2 shows these results. This indicates that 37.5° C. is the optimum temperature. The organisms lived three hours when the temperature reached 42° C. and died at the end of eight hours at a temperature of 41° C.

TABLE 2.—*Length of Life at 37.5° C.*

Hours	6	10	20	28	44	50	54	66	72	73	74	75
Stool No. 1 + Ringer's	+	+	+	+	+	+	+	−	−	−	−	−
Stool No. 2 + Ringer's	+	+	+	+	+	+	+	−	−	−	−	−
Stool No. 3 + Locke's	+	+	+	+	+	+	−	−	−	−	−	−
Stool No. 4 + Locke's	+	+	+	+	+	+	−	−	−	−	−	−
Stool No. 5 + Normal saline	+	+	+	+	+	−	−	−	−	−	−	−
Stool No. 6 + Normal saline	+	+	+	+	+	+	−	−	−	−	−	−
Stool No. 7 + Tap water	+	+	+	+	+	+	+	+	+	+	+	−
Stool No. 8 + Tap water	+	+	+	+	+	+	+	+	+	+	−	−
Total	8	8	8	8	8	7	4	2	2	2	1	0

The relation of the length of life to the temperature can be seen very clearly in Graph 2.

When the organisms observed were not moving and could not be stimulated to activity by warming the slide, they were considered dead.



GRAPH 2. Length of life in fecal material.

Living organisms are usually very actively beating their cirri. Dead organisms can readily be distinguished by their brown color.

DISCUSSION

While the only medium that was used successfully for the cultivation of *Troglodytella abrassarti* was chimpanzee fecal extract, several others that were tried might be modified to produce a suitable culture medium. The most promising of these is Ringer's solution + 10 per cent serum.

Charcoal, added to one series of tubes on the theory that it would inhibit bacterial growth, had no helpful effect. One tube containing charcoal seemed to be aided, but the others showed no improvement. The same was true where white of egg was added.

The ciliates were kept in culture for eight days in the same medium as that used by Nelson (1932) but no multiplication appeared to be taking place for the tubes lasted only seven or eight days despite repeated subcultures. When no subcultures were made and no rice starch was added, the infusorions remained viable for the same length of time but did not appreciably change in numbers. They merely lived in the culture medium for seven or eight days and then died. This leads the writer to the conclusion that Nelson, although keeping the organisms alive, probably did not cultivate them. Chimpanzee fecal extract showed an entirely different picture in that the counts were much higher on the second day than on the first, and on the third than on the second, proving beyond all reasonable doubt that multiplication actually occurred—and very rapidly.

It is difficult to state why these ciliates, which occur in the same host as *Balantidium coli,* are so difficult to cultivate in media in which *Balantidium* grows and multiplies. It may be connected, in some way, to the fact that *Balantidium coli* has such a loose host-parasite specificity, while that of *Troglodytella* is very rigid. Brumpt and Joyeux (1912) attempted several cross-infection experiments but failed and Reichenow (1917 and 1920) notes that *Troglodytella abrassarti* occurred only in chimpanzee while *Troglodytella gorillae* appeared only in gorillas, even though they both lived in the same locality and had the same type of food and similar habits. This rigid host-parasite specificity may help to explain the difficulty in cultivating these ciliates.

In the experiments to determine the length of life of the organisms in the feces, the temperature of 30° C. permitted a maximum length of life of twenty-eight hours when the fecal material was not permitted to dry out. Since chimpanzees and gorillas live, as they do, in tropical countries, where the feces are often deposited in moist places, they tend to increase the length of life in the stool up to the maximum of twenty-four to twenty-eight hours. So, *assuming* that no cysts are formed, since none have been described, and the organisms are so closely related to the ruminant infusoria, which produce no cysts but are transmitted by saliva (Becker and Hsuing, 1929), the possibility of transmission of the vegetative forms is greatly increased due to the comparatively long length of life of the organisms in the stool. However, it is not known whether or not these organisms, in the vegetative condition, are able to pass through the stomach and small intestine to the large intestine of the chimpanzee

or gorilla unharmed. If they cannot, there only remains the possibility of a rare cyst which produces the infection.

SUMMARY

1. *Troglodytella abrassarti* has been cultivated in an extract, in Ringer's solution, of chimpanzee fecal material and counts have been made to prove that multiplication actually takes place. These counts show a rapid increase in the number of organisms on the second and third days, then a gradual decrease until all are dead.

2. It was found that these organisms live for longer periods of time in chimpanzee fecal extract than in any other of the various media tried, when no subcultures are made and no food is added to the cultures.

3. The optimum temperature of these infusorions is 37.5° C. but they will live in moistened feces for twenty-eight hours at 30° C. This temperature is close to that at which the chimpanzees live and may help to explain the transmission of the organism. Temperatures above 40° C. or below 30° C. are very detrimental to these ciliates.

LITERATURE CITED

Atchley, F. O., and Swezey, W. W. 1934.—A Method for the Enumeration of Ciliate Protozoa. Trans. Am. Mic. Soc., 53: 35–39.

Becker, Elery R., and Hsuing, T. S. 1929.—The Method by which Ruminants Acquire their Faun of Infusoria, and Remarks concerning Experimentation on the Host-parasite Specificity of these Protozoa. Proc. Nat. Acad. Sc., 15: 684–690.

Brumpt, E., and Joyeux, Ch. 1912.—Sur un Infusoire Nouveau Parasite du Chimpanzé *Troglodytella abrassarti* n. g., n. sp. Bull. Soc. Path. Exot., 5: 499–503.

Cleveland, L. R., and Collier, Jane. 1930.—Various Improvements in the Cultivation of *Entamoeba histolytica.* Am. Jour. Hyg., 12: 606–613.

Nelson, E. C. 1932.—The Cultivation of a Species of *Troglodytella,* a large Ciliate from the Chimpanzee. Science, 75: 317–318.

Reichenow, E. 1917.—Parasites de la Sangre y del Intestino de los Monos Antropomorfos Africanos. Bol. R. Soc. Esp. de Historia Natural, 17: 312–332.

1920.—Den Wiederkaüer—Infusorien verwaudte Formen aus Gorilla und Schimpanse—Arch. f. Protistenk., 41: 1–33.

Schumaker, Eugene. 1931.—The Cultivation of *Balantidium coli.* Am. Jour. Hyg., 13: 281–295.

St. John, J. H. 1932.—A New Medium for the Cultivation of *Endamoeba histolytica.* Am. Jour. Trop. Med., 12: 301.

Stoll, Norman R., and Hausheer, W. C. 1926.—Accuracy in the Dilution Egg Counting Method. Am. Jour. Hyg., 6: 80–133.

Tanabe, Misao, and Chiba, Eiichi. 1928.—A New Culture Medium for *Entamoeba histolytica.* Acta. Med. in Keijo, 11–1–4.

A DESCRIPTION OF *CERCARIA FLEXICORPA* N. SP.[1]

Willis W. Collins

Cercaria flexicorpa n. sp. was found in the vicinity of Stillwater, Oklahoma, during the summer of 1932. A study of 5,000 specimens of *Helisoma trivolvis* (Say)[2] revealed 25 infected with this species. The type and one cotype specimen, mounted *in toto* on separate slides, have been deposited in the Parasite Collection (Nos. 337 and 338, respectively), Museum of Zoology, University of Michigan.

Four of the infected snails lived in the laboratory, producing cercariae continually, for over 11 months.

In behavior the present species resembles *Cercaria bessiae* Cort and Brooks (1928, 204). Most of the free life is spent suspended head downward in the water with the tail-stem fully extended, the furcae spread at an obtuse angle to each other, and the anterior third of the body doubled back on itself ventrally (Figs. 2 and 3). When a number of cercariae are observed in water only a few will be in motion. The individual cercaria swims infrequently, in short sporadic dashes, tail forward. It is viable for a period of 24 to 30 hours after escaping from the snail host.

The cercariae develop in typical thread-like sporocysts found in tangled masses in the liver of the snail.

Studies were made of both living and preserved material. The study of living animals yielded more information. For permanent mounts specimens were fixed in hot 5 per cent formalin and stained with borax carmine.

Specific Description

Measurements, in microns, of 20 preserved specimens of *C. flexicorpa:* total length 399 to 607 (average 516); body length, 107 to 182 (average 159); body width, 19 to 32 (average 26); tail-stem length, 156 to 227 (average 188); tail-stem width, 13 to 35 (average 22); length of furcae, 136 to 198 (average 169). Body shape, essentially as outlined for *C. hamata* Miller (1926). Body spination not extending caudad to pharynx. Solid piercing spines arranged in two rows anterior and dorsal to mouth; anterior row slightly curved, convex side forward, posterior row more or less straight. Tail-stem, attached terminally, diameter uniform.

Digestive system, single median capillary tube leading from minute ventroterminal mouth, terminating in very small vesicle partially embedded in posterior end of rudimentary pharynx. Small pharynx, difficult to see in living specimens, apparently non-muscular, represented in preserved specimens by compact group of nuclei averaging 10 microns in diameter.

---

[1] A contribution from the Zoology Laboratory, Oklahoma Agricultural and Mechanical College, prepared under the direction of R. Chester Hughes, Assistant Professor of Zoology.

[2] Identified by Calvin Goodrich, of the Museum of Zoology, University of Michigan.

Ten pairs of flame cells (Fig. 1), eight in body proper and two in tail-stem. Excretory vesicle longitudinally bipartite. Openings of furcal excretory ducts on anterior surfaces, slightly proximal to middles of furcae.

Three pairs of penetration gland cells partially divided by so-called "anterior cell mass" into two contiguous tandem groups; anterior group regularly containing two cells, posterior group, four; content of cells coarsely granular staining readily with neutral red, exhibiting bright yellowish red color. Ducts easily traceable throughout entire lengths, in triangular arrangement in cross-section. Two lateral groups of ducts, practically contiguous for greater part of lengths, diverge laterally around pharynx to enter anterior organ (Fig. 1).

Two prominent compact groups of cells in posterior third of body, the anterior mass between first and second pairs of penetration glands, the much larger posterior mass between penetration glands and urinary bladder. [Because of uncertainty relative to their significance Miller (1926) refers to similar groups of cells in *C. hamata* as anterior and posterior cell masses. Like structures in *C. bessiae* are regarded (Cort and Brooks, 1928, 205) as a rudimentary ventral sucker and a genital primordium, respectively.]

Tail-stem—no caudal gland cells; four longitudinal "muscle bands" as in *C. hamata*; sensory hairs not constant in occurrence or arrangement, sometimes entirely absent. Furcae laterally flattened, with thin edges, without noticeable fin-folds, six longitudinal rows of spines on each surface.

In the posterior part of oral sucker are four nuclei (Fig. 4 *nv* and *nd*), which I believe pertain to four head gland cells, located between the two lateral groups of penetration gland ducts. These nuclei are conspicuous in living material when stained with neutral red, to which they respond more quickly than other parts of the body. In stained preserved material they are easily distinguishable from the nuclei of parenchymal cells, by their greater size, brighter coloration and isolated position. The outlines of these cells, however, are very indistinct and their boundaries only faintly suggested. It may be that they comprise a syncytium. They are arranged in pairs, two dorsally and two ventrally. They are longitudinally elongate, the dorsal cells being more slender with their nuclei more posterior and mesial in position. When stained with neutral red (*in vivo*) the finely granular content has a faint pink color, easily distinguishable from the coarsely granular and more brightly colored fluid of the penetration glands. It was not determined whether the head glands have external openings.

C. flexicorpa n. sp. a pharyngeal monostome longifurcate cercaria, closely resembles four species that have been described from the United States. They are *Cercaria rhabdocaea* Faust (1919), *Cercaria multicellulata* Miller (1923 and 1926), *Cercaria hamata* Miller (1923 and 1926), and *Cercaria bessiae* Cort and Brooks (1928). The five species are similar in lacking a functional ventral sucker and in having three pairs of penetration gland cells, the same type of rudimentary digestive system, and similar spination. *C. flexicorpa* differs from *C. rhabdocaeca* in the uniformly ovoidal shape of the penetration organ, the contiguity of the two groups of penetration glands, the relatively shorter length of the tail-stem, the shortness of the caecum, and the location of the furcal ex-

cretory pores on the anterior surfaces of the furcae; from *C. bessiae* in the absence of eyespots, the number and arrangement of flame cells, the form of the excretory bladder, and the arrangement of the penetration glands and ducts, especially the openings of the latter to the exterior; from *C. multicellulata* in having the anterior end bent in a hook while resting in the water, the presence of a distinctly visible pharynx, the absence of eyespots, the apparent absence of fin-folds on the furcae, and the lack of caudal bodies.

C. flexicorpa differs from *C. hamata* as described by Miller (1926, 55) in the following features. The average measurements are smaller than those given for *C. hamata* which may be due in part to methods of fixation. It is perhaps significant, however, that the tail-stem is disproportionately short. The body proper is devoid of spines posterior to the oral sucker. The ducts of the peneration glands are not difficult to see individually. The piercing spines are not "arranged in a crescent." The pharynx shows no evidence of muscularity. The so-called "cell masses" do not appear to be joined by lateral connections, nor is the "anterior cell mass" provided with lateral extensions that run forward to meet in the median plane. The "anterior cell mass" separates the penetration glands into two groups the anterior of which *regularly* contains two cells and the posterior, four cells. The excretory bladder is not enlarged anteriorly. There are no caudal gland cells. The flame cells in the tail-stem are not particularly difficult to observe. Sensory hairs on the tail-stem are apparently not of constant occurrence or regular arrangement.

Head glands are not mentioned or figured in the descriptions of any of the four above-mentioned species with which *C. flexicorpa* is compared.

References

Cort, W. W., and S. T. Brooks. 1928.—Studies on the holostome cercariae from Douglas Lake, Michigan. Trans. Amer. Micros. Soc., 57: 179–221.

Faust, E. C. 1919.—The excretory system in Digenea. II. Observations on the excretory system in distome cercariae. Biol. Bull., 36: 322–339.

Miller, H. M., Jr. 1926.—Comparative studies on furcocereous cercariae. Ill. Biol. Mon., 10 (3), 112 p.

Explanation of Figures

All figures concern *Cercaria flexicorpa,* n. sp.

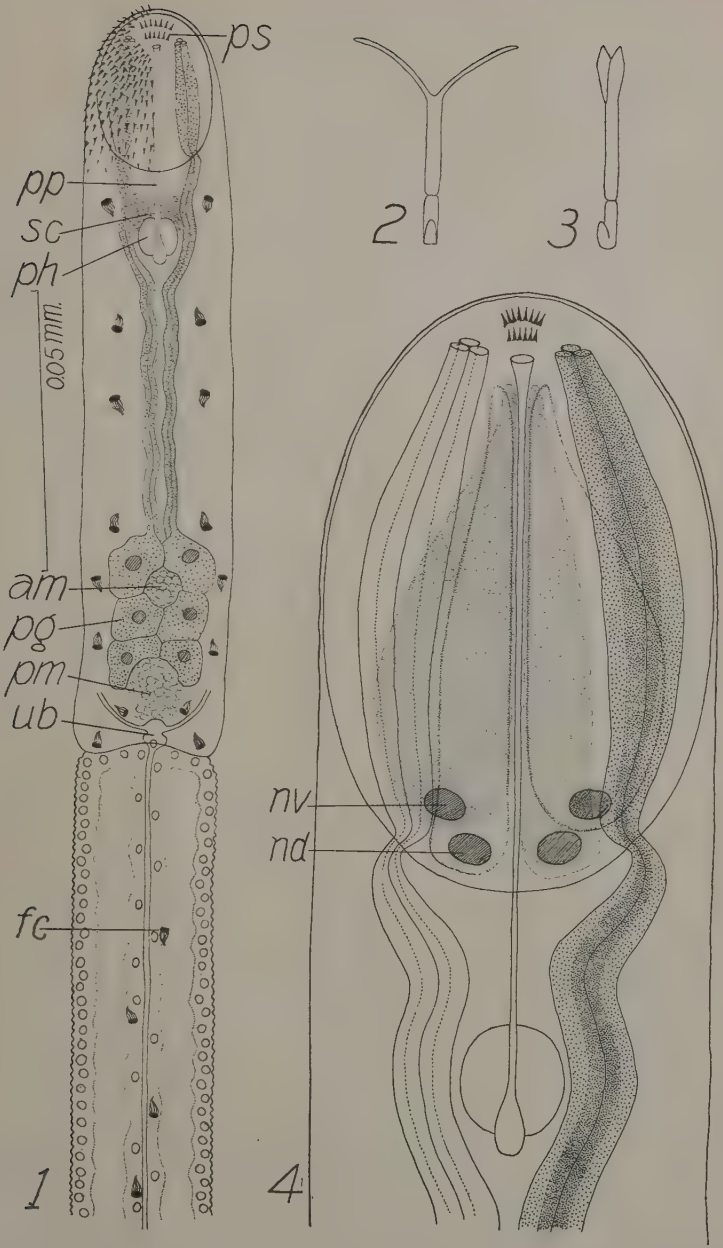
Fig. 1. Ventral view. Outline drawn from a preserved specimen with the aid of a camera lucida; finer details added from observations on living animals.

Fig. 2 and 3. Free-hand sketches of living cercariae to show the natural resting position of the animal in water.

Fig. 4. Enlarged free-hand sketch of oral sucker showing details of head glands in ventral view. The left dorsal and right ventral head glands are shown in stipples.

Abbreviations

am, anterior cell mass; *fc,* flame cell; *nd,* nucleus of dorsal head gland; *nv,* nucleus of ventral head gland; *pg,* penetration gland; *ph,* pharynx; *pm,* posterior cell mass; *pp,* prepharynx; *sc,* suprapharyngeal commissure; *ub,* urinary bladder.


ps
pp
sc
ph
0.05 mm.
am
pg
pm
ub
fc
1
2
3
nv
nd
4

AN INVESTIGATION OF POSSIBLE VECTORS OF *WUCHERERIA BANCROFTI* (COBBOLD) IN BAHIA, BRAZIL[1]

NELSON C. DAVIS, M.D.

(From the Yellow Fever Laboratory of the International Health Division of The Rockefeller Foundation, Bahia, Brazil)

The laboratory occupied by the Yellow Fever Service at São Salvador, Bahia, Brazil, is situated at the edge of a district called Mont Serrat. This section is reported to have a higher filarial index than any other part of the city. Local medical men have carried out a number of filarial surveys. The most recent of these was made by Magalhães (1) in 1928. He examined the blood of three hundred persons who had resided in the district for three years or more; of these, thirty-seven, or 12.33 per cent, were found to be infected. Among an additional twenty persons who had elephantiasis or other clinical manifestations attributable to filariasis, five were found infected; among thirty persons who had lived in the district for less than three years, none was infected. In the grand total of 350 examined, therefore, forty-two, or 12 per cent, showed microfilariae in the blood. Magalhães cites figures from surveys preceding his own.

Since several laboratory employees with their families reside in Mont Serrat, or nearby, it seemed worth while to know whether any of them had become infected with filariae. Sr. Antonio S. Cabral, assistant in the laboratory, examined approximately one hundred thick blood smears from eighty persons who had requested examinations. These persons were laboratory employees, members of their immediate families, and a few other relatives and friends who were interested. In order to cause a minimum of inconvenience the specimens were taken at about 10 p. m.; this early hour probably accounted in part for the low index. Only three positive smears were found, two from children, and one from the wife of a laboratory employee. The adult showed a much heavier infection than did the children; she was used in all the subsequent mosquito feeding experiments.

This woman is about forty-four years old and has lived in the infested district for fifteen years or more. She has no elephantiasis and no definite history of lymphangitis, but suffers from occasional attacks of unexplained fever. For a small compensation she readily agreed to allow mosquitoes to feed on her arms. Cages containing the insects were sent home with her husband at the end of the day's work. Feedings

[1] The studies and observations on which this paper is based were conducted with the support and under the auspices of the International Health Division of The Rockefeller Foundation.

were supposed to take place just before the hour of retiring, usually at about 10 p. m. Since these feedings were not under the direct observation of the writer, it was deemed advisable always to send some *Culex fatigans* to take blood as controls. As nearly as can be determined from the dissections, the feedings were always carried out conscientiously.

All the mosquitoes used for the experiments were bred from larvae, with the exception of the *Mansonia justamansonia*. The latter were caught as adults in an uninhabited marsh about four miles outside the city. Insects were prepared for examination by teasing them with fine needles in physiological saline solution. No sections were made. It is realized that probably some infections were overlooked because of the technique employed and because mosquito abdomens were not examined routinely. The greatest number of worms found in any dissection was fifteen. The average number in positive dissections of all species was 2.5. No quantitative counts of microfilariae were made; occasional blood smears taken as checks showed moderate numbers of parasites. The morphology of the microfilariae and of the developmental stages in mosquitoes agreed with that of *Wuchereria bancrofti*. Infective forms reached a maximum length of about 1.5 mm.

Experiments were carried out between October 10, 1932, and April 19, 1933, that is, in spring, summer, and early autumn, south of the equator. Slight differences in the rapidity of growth of the worms in *Culex* were noted as the seasons changed. The first proboscis infection in *C. fatigans* was found on the seventeenth day after the ingestion of microfilariae; at the height of summer a head infection was found on the twelfth day, and proboscis infections on the thirteenth day. In the last experiment, proboscis infections were not found until the fifteenth day. These differences may perhaps have been associated with changing meteorological conditions, but it is felt that no stress can be laid upon the findings from a statistical point of view because so few mosquitoes were dissected upon any given day.

When the infection experiments were begun, it was decided not to go too far afield in the selection of mosquitoes for study. It was felt that the number of vector species to be found in the city was undoubtedly rather limited. In Mont Serrat the mosquitoes under suspicion were: *Culex fatigans, Culex nigripalpus, Aëdes scapularis, Aëdes taeniorhynchus, Anopheles albitarsis, Anopheles bachmanni, Anopheles tarsimaculatus,* and *Anopheles argyritarsis,* the last two being relatively scarce. Of late years the breeding of *Aëdes* (*Stegomyia*) *aegypti* has been held in check by the yellow fever control service. Table 1 summarizes experiments with most of the species named above.

Proboscis infections were found in *Culex fatigans* Wiedemann, in *Mansonia* (*Rhynchotaenia*) *justamansonia* (Chagas), and in *Anopheles* (*Nyssorhynchus*) *albitarsis* Arribálzaga. The last-named species did

not appear to be a highly efficient host; development of the filariae in this species almost invariably lagged slightly behind that in *Culex* controls. In the thorax of one *Anopheles bachmanni* on the fourteenth day a filaria was found which had apparently reached the infective stage. Development occasionally proceeded to an advanced stage in *Culex nigripalpus*; whether this species may ever become infective is problematical. No development of filariae occurred in *Aëdes scapularis* and in *Aëdes taeniorhynchus*. Invasion of the thorax and slight metamorphosis took place in *Aëdes fluviatilis* and in *Aëdes aegypti*. Although a live filaria was seen in the thorax of the latter species as late as the eleventh day, no development was noted greater than that found on the third or fourth day in *Culex* controls; in many cases the microfilariae remained unmodified.

In addition to the dissections summarized in Table 1, four *Anopheles tarsimaculatus* were examined on the fifteenth day. One thorax was found to contain a single filaria, alive, but very much retarded in development. There were also dissected four *Mansonia* (*Mansonia*) *indubitans*, one on the eleventh day and three on the fourteenth day. All were negative.

DISCUSSION

Culex fatigans is recognized as an efficient host for *Wuchereria bancrofti* wherever the microfilariae have a nocturnal periodicity. Ever since the early work of Low (2), only partial metamorphosis, followed by degeneration, has usually been found to take place in *Aëdes aegypti*. There are at least two observations to the contrary. Flu (3) thought that the species was a vector in Surinam. More recently Hicks (4) obtained one experimental proboscis infection on the sixteenth day; he regarded this as a very exceptional finding.

There appear to have been no previous attempts to infect *Aëdes scapularis, Aëdes fluviatilis, Culex nigripalpus, Anopheles bachmanni*, or *Mansonia justamansonia*. Cesar Pinto (5) states that partial development takes place in *Aëdes taeniorhynchus*, but he does not cite the experimental work upon which his statement is based.

It is very probable that all members of the *Nyssorhynchus* group of anopheline mosquitoes will permit development of filarial larvae. Vincent (6) found a rapid metamorphosis in *Anopheles albimanus*. Unfortunately the last of his experimental batch died on the twelfth day, just when it seemed that invasion of the proboscis was imminent. Partial development is said to occur in *Anopheles argyritarsis* (Cesar Pinto (5), Manson-Bahr (7), Edwards (8); but references to the experimental work with this species and *Wuchereria bancrofti* are not cited. The writer found filarial infections, including a proboscis infection, of *Anopheles darlingi* in nature in Belém, Pará (9). In a list of mosquitoes

TABLE I.—*Results of Infection Experiments with Bahian Mosquitoes*

Species of Mosquito	Mosquito Lot Numbers	Days after Feeding on Filaria Carrier	Mosquito Abdomens			Mosquito Thoraces			
			Examined	With Micro-filariae Present	With Live Micro-filariae	Examined	With Larvae Present	With Live Larvae	With Larvae Apparently Mature
Culex fatigans	774, 789,	1–4	27	13	9	53	28	26	0
	812, 830,	5–9	—	—	—	64	35	33	0
	836, 839,	10–21	—	—	—	242	88	83	59
	848	1–21	27	13	9	359	151	142	59
Culex nigripalpus	794, 803	1–4	13	5	4	13	5	5	0
		5–9	—	—	—	29	7	4	0
		10–15	—	—	—	55	3	1*	0
		1–15	13	5	4	97	15	10	0
Aëdes (Stegomyia) aegypti	775	1–4	10	0	0	20	12	10	0
		5–9	—	—	—	31	10	9	0
		10–23	—	—	—	132	35	2*	0
		1–23	10	0	0	183	57	21	0
Aëdes taeniorhynchus	790, 793	1–4	12	5	4	15	0	0	0
		5–9	3	0	0	8	0	0	0
		10–16	—	—	—	26	1†	0	0
		1–16	15	5	4	49	1	0	0
Aëdes scapularis	837, 843	1–4	16	6	4	16	0	0	0
		5–9	—	—	—	14	0	0	0
		10–15	—	—	—	21	0	0	0
		1–15	16	6	4	51	0	0	0
Aëdes fluviatilis	840, 847	1–4	6	1	0	6	2	0	0
		5–9	—	—	—	6	2	2	0
		10–12	—	—	—	14	3	0	0
		1–12	6	1	0	26	7	2	0
Anopheles bachmanni	804, 813,	1–4	—	—	—	1	0	0	0
	818, 829	5–9	—	—	—	1	0	0	0
		10–15	—	—	—	8	2	2	1
		1–15	—	—	—	10	2	2	1
Anopheles albitarsis	804, 813,	1–4	4	3	2	4	3	3	0
	818, 829,	5–9	—	—	—	2	1	1	0
	833, 838,	10–16	—	—	—	38	15	15	10‖
	841	1–16	4	3	2	44	19	19	10
Mansonia justamansonia .	842	1–4	2	2	2	4	3	2	0
		5–9	—	—	—	2	2	2	0
		10–15	—	—	—	11	5	5	4
		1–15	2	2	2	17	10	9	4

* Larva between .9 mm. and 1.0 mm. in length; almost mature.
† Movements were detected in both larvae (tenth and eleventh days).
‡ Two badly degenerated forms present; evidently had not developed at all after invading thorax.
‖ In two instances larvae had penetrated coxae.
§ In one of these dissections the proboscis and palps had been loosened from the head; a filaria was found industriously penetrating a palp.

TABLE I.—(*Continued*)

Species of Mosquito	Mosquito Heads and Proboscides				First Head of Proboscis Infection. Days after Feeding on Carrier	Total Mosquitoes Examined	Total Positive	*Culex fatigans* Controls	
	Examined	Heads with Larvae	Proboscides with Larvae	Total Positive				Heads and Proboscides Examined	Number of *Culex* with Larvae in Head or Proboscis
Culex fatigans	—	—	—	—					
	—	—	—	—					
	221	26	36	55					
Culex nigripalpus	221	26	36	55	12	359	183		
	—	—	—	—					
	—	—	—	—					
	45	0	0	0					
Aëdes (Stegomyia) aegypti	45	0	0	0	—	43	23	31	9
	—	—	—	—					
	—	—	—	—					
	33	0	0	0					
Aëdes taeniorhynchus	33	0	0	0	—	183	57	32	5
	—	—	—	—					
	—	—	—	—					
	2	0	0	0					
Aëdes scapularis	2	0	0	0	—	49	6	65	24
	—	—	—	—					
	—	—	—	—					
	15	0	0	0					
Aëdes fluviatilis	15	0	0	0	—	51	6	45	13
	—	—	—	—					
	—	—	—	—					
	14	0	0	0					
Anopheles bachmanni	14	0	0	0	—	26	8	36	3
	—	—	—	—					
	—	—	—	—					
	8	0	0	0				See summary of	
Anopheles albitarsis	8	0	0	0	—	10	2	*C. fatigans* above	
	—	—	—	—					
	—	—	—	—					
	36	3§	1	4				See summary of	
Mansonia justamansonia ..	36	3	1	4	15	44	19	*C. fatigans* above	
	—	—	—	—					
	—	—	—	—					
	10	3	5	5					
	10	3	5	5	15	17	12	19	2

* Larva between .9 mm. and 1.0 mm. in length; almost mature.
† Movements were detected in both larvae (tenth and eleventh days).
‡ Two badly degenerated forms present; evidently had not developed at all after invading thorax.
‖ In two instances larvae had penetrated coxae.
§ In one of these dissections the proboscis and palps had been loosened from the head; a filaria was found industriously penetrating a palp.

which permit complete development of filarial worms, Edwards (10) in a recent publication includes both *Anopheles* (*Nyssorhynchus*) *albitarsis* and *A.* (*Nys.*) *tarsimaculatus*. No references are given to the original work. Members of the *Nyssorhynchus* group should be considered as probable vectors, accessory to the usual *Culex* host.

The chief transmitter in Bahia, Brazil, is without doubt *Culex fatigans*.

SUMMARY

1. Experimental proboscis infections with *Wuchereria bancrofti* (Cobbold) were obtained in the following mosquitoes: *Culex fatigans* Wiedemann, *Mansonia* (*Rhynchotaenia*) *justamansonia* (Chagas), and *Anopheles* (*Nyssorhynchus*) *albitarsis* Arribálzaga.

2. Advanced development of larvae took place occasionally in *Anopheles* (*Nyssorhynchus*) *bachmanni* Petrochi and in *Culex nigripalpus* Theobald. Retarded development was also noted in one specimen of *Anopheles* (*Nyssorhynchus*) *tarsimaculatus* Goeldi.

3. A slight degree of development, followed by degeneration, occurred in *Aëdes* (*Stegomyia*) *aegypti* (Linnaeus) and in *Aëdes* (*Ochlerotatus*) *fluviatilis* (Lutz).

4. No metamorphosis was noted in *Aëdes* (*Ochlerotatus*) *taeniorhynchus* (Wiedemann) or in *Aëdes* (*Ochlerotatus*) *scapularis* (Rondani). Invasion of the thorax occurred only once in *Aëdes taeniorhychus* and never in *Aëdes scapularis*.

REFERENCES

1. Magalhães, Gualberto Vicente de Paulo. 1928.—Bancroftose na Bahia. Thesis for the doctorate, presented to the Faculty of Medicine of Bahia.
2. Low, George C. 1901.—The Development of Filaria Nocturna in Different Species of Mosquitoes. *Brit. Med. Jour.*, 1 : 1336–1337.
3. Flu, P. C. 1928.—Verslag van een studiereis naar Suriname (Nederlandsch Guyana), Sept. 1927–Dec. 1927, en beschouwingen dienaangaande. *Acta Leidensia Scholae Med. trop.*, 3 : 1–192. Summarized in *Rev. Appl. Ent.*, Series B, 1929, 17, 192.
4. Hicks, E. P. 1932.—The Transmission of *Wuchereria bancrofti* in Sierra Leone. *Ann. Trop. Med. & Parasit.*, 26 : 407–422.
5. Pinto, Cesar. 1930.—Tratado de Parasitologia. Vol. IV. Arthrópodes Parasitos e Transmissores de Doenças. Bibliotheca Scientifica Brasileira. Pimento de Mello & Co., Rio de Janeiro. Tomo II, page 704.
6. Vincent George A. 1902.—Observations on Human Filariasis in Trinidad, W. I. *Brit. Med. Jour.* (*Jan. 25*), 1 : 189–190.
7. Manson-Bahr, Philip H. Ed. 1929.—Manson's Tropical Diseases. A Manual of the Diseases of Warm Climates. Ninth Edition, Revised. New York, William Wood and Co.
8. Edwards, F. W. 1922.—The Carriers of *Filaria bancrofti*. *Journ. Trop. Med. & Hyg.*, *25*, 168–170.
9. Davis, N. C. 1931.—A Note on the Malaria-Carrying Anophelines in Belém, Pará, and in Natal, Rio Grande do Norte, Brazil. *Rivista di Malariologia*, 10, 43–51.
10. Edwards, F. W. 1932.—Genera Insectorum (P. Wytsman). 194me Fascicule. Diptera, Fam. Culicidae. 258 p. Louis Desmet—Verteneuil, Brussels.

THE NEMATODA AS PARASITES OF AMPHIBIA. II.[1]

A. C. WALTON,

Knox College

The first paper of this series dealt with the members of the superfamily Ascaroidea. The present article includes those forms belonging to the superfamilies Filarioidea, Spiruroidea, Strongyloidea, Dioctophymoidea and Trichinelloidea as parasitic in amphibian hosts.

A. FILARIOIDEA

This order is represented by 15 species of the genus *Filaria*, by 7 species of the genus *Foleyella*, and by two species of the genus *Icosiella* as parasites of amphibia. Two microfilariids of undetermined relationship are also reported from amphibian hosts. Hosts from all of the Continents are represented.

I. Filariidae. Two genera of the subfamily Filariinae and one of the subfamily Setariinae are reported.

A. Filariinae

1. *"Filaria"* (s. l.)

a. *F. appendiculata* Schneider, 1866. From intestinal muscles of *Triturus vulgaris*—Europe. (This is apparently a *nomen nudem* as Schneider figures, but does not describe any form under this name.)

b. *F. bufonis* M. C. V. Molin, 1858 (=*F. eupemphigis-marmorati* Molin, 1858). A larval form from abdominal cavity of *Eupemphix marmoratus*—Brazil. (undescribed)

c. *F. cingula* v. Linst., 1902. Females only, from skin of *Cryptobranchus alleganiensis*—U. S. A., and *Megalobatrachus japonicus*—Japan.

d. *F. cochleata* Railliet, 1916 (=*F. spiralis* Oerley, 1882—renamed). From stomach cysts of *Heleioporus albopunctatus*—Australia. (undescribed)

e. *F. columbi* R. Blanchard, 1905. Larval forms from blood of "Toad"—Columbia, S. America.

f. *F. jubae* Seurat, 1916 (=*F. rubella* Rud., 1819 *ex. p.* (*vide* Seurat. 1917); =*Spiroptera bufonis* Stoss., 1900). From abdominal cysts in *Bufo bufo, Rana esculenta* and *R. temporaria*—Europe.

g. *F. parva* Polonio, 1859. From throat muscle cysts in *Bufo viridis*—Italy. (undescribed)

h. *F. rubella* Rud., 1819 (=*F. ranae-esculentae* Val., 1841; =*Trichina ranae-esculentae* Chaussat, 1850). From cysts in *Rana esculenta* and *R. temporaria*—Europe.

i. *F. unguiculata* v. Linst., 1906. From *Bufo viridis*—Europe (Corfu). (undescribed)

---

[1] Contribution from the Biological Laboratories of Knox College, No. 46. This paper was received for publication on April 17, 1933, and therefore does not include material published since that date.

j. *F.* sp.? Leidy, 1852. From *Rana pipiens*—U. S. A. (undescribed)
k. *F.* sp.? v. Linst., 1889. From *Rana temporaria*—Europe. (undescribed)
l. *F.* sp.? Schn., 1866. From *Triturus vulgaris*—Europe. (undescribed)
m. *F.* sp.? Plimmer, 1912. From *Bufo marinus*—S. America. (undescribed)
n. *F.* sp.? Léger, 1918 (=*Microfilaria* sp.? Léger, 1918). From *"Bufo"*—Europe. (undescribed)
o. *F.* sp.? Walton, 1927 (=*Strongylus* sp.? Call, 1887). From cysts in *Ambystoma tigrinum*—U. S. A. (undescribed)

TABLE I.—*"Filaria"* s. l.

All measurements in millimeters	*F. cingula* female	*F. columbi* larvae	*F. jubae* larvae	*F. rubella* female
Length	200	0.075–0.09	38.3 –43.8	27–120
Width	0.53	0.005	0.816– 0.85	0.27
Pharynx length	0.375	?	1.57	?
Esophagus length	15	?	1.98 – 2.05	?
Head-nerve ring distance	?	?	0.42	?
Head-excretory pore distance	?	?	0.445– 0.475	?
Anus-tail distance	terminal	?	0.12 – 0.132	
Head-vulva distance	post-esophageal			0.18 0.83
Egg sizes	viviparous larva, 0.014 × 0.33			0.22 × 0.22

2. *Foleyella*

a. *F. americana* Walton, 1929. From thoracic cavity of *Rana pipiens* —U. S. A.
b. *F. convoluta* (Molin, 1858) Trav., 1928 (=*Filaria ranae* M. C. V. Molin, 1858; =*Filaria convoluta* Molin, 1858). From thoracic cavity of *Hyla faber, Leptodactylus ocellatus, L. pentadactylus* and *L. typhonius*—Brazil.
c. *F. duboisi* (Gedoelst, 1916) Yorke & Maplestone, 1926 (=*Filaria duboisi* Gedoelst, 1916). From thoracic cavity of "Frog" and "Toad"—Belgian Congo, Africa.
d. *F. leiperi* (Railliet, 1916) Yorke & Maplestone, 1926 (=*Filaria bufonis* Leiper, 1908; =*F. leiperi* Raill., 1916; =*F.* sp.? Bouilliez, 1916; =*Microfilaria bufonis* Leb. & Ring., 1910). From "Connective tissue" in *Bufo regularis*—Sudan, Africa.
e. *F. ranae* Walton, 1929. From thoracic cavity of *Rana catesbiana* —U. S. A.
f. *F. scalaris* Trav., 1928. From sub-lingual tissues in *Leptodactylus ocellatus*—Brazil.
g. *F. vellardi* Trav., 1928. From thoracic cavity of *Bufo marinus*—Brazil.

Foleyella americana Walton, 1929 (Text-fig. 1).

Only the female of this species has been described. The following is a description of the male, recovered from the thoracic cavity of a Wisconsin specimen of the type host, *Rana pipiens*.

Male. Mouth opening surrounded by ten papillae in two circles, the outer of four and the inner of six members. Length, 23 mm. Greatest diameter, 0.45 mm. Length of anterior portion of esophagus, 0.33 mm.; of the posterior thicker

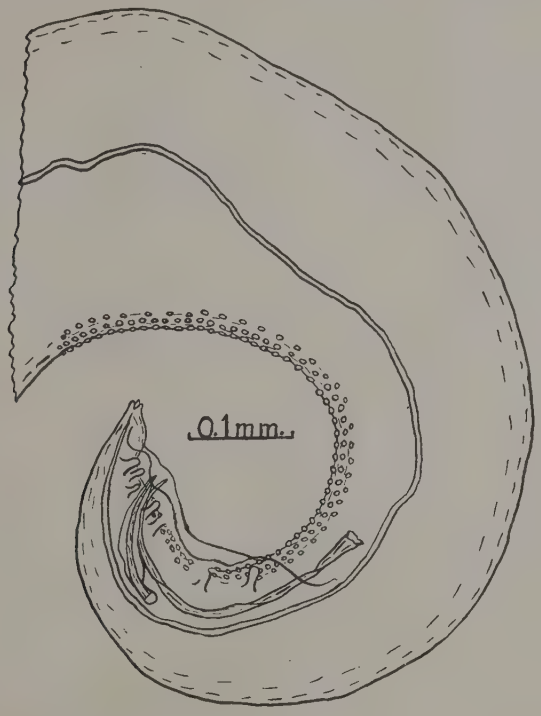


FIGURE 1

portion, 2.2 mm. Head-nerve ring distance, 0.2 mm. Cloaca-tail distance, 0.075 mm. The pre-cloacal area covered by a narrow ventral band of cuticular bosses arranged in definite rows. Caudal papillae pedunculated, 4 pairs pre-cloacal and 3 pairs post-cloacal in position. Right spicule short, 0.11 mm., and alate; left spicule longer, 0.4 mm., and slightly twisted.

Type specimen deposited in the Helminthological Collection of the U. S. National Museum.

B. Setariinae

1. *Icosiella*

a. *I. neglecta* (Dies., 1851) Seurat, 1917 (=*Filaria rubella* Rud., 1819 *ex. p.*; =*F. ranae-esculentae* Rud., 1819; =*F. ranae-esculentae* Val., 1841 *ex. p.*; =*F. ranae-esculentae* Mathis & Léger, 1912—from blood). From sub-cuticular cysts of *Rana esculenta* and *R. temporaria*—Europe; from *R. ridibunda*—Africa; and from *R. tigerina*—Tonkin, Asia. Length: Male, 7.2–11.04 mm.; female, 20.95 mm. Width: Male, 0.18 mm.; female, 0.28 mm. Length of muscular esophagus: Male, 0.45 mm.; female, 0.41 mm. Length of glandular esophagus: Male, 5.7 mm.; female, 5.3 mm. Head-nerve ring distance: Male, 0.33 mm.; female, 0.3 mm. Anus-tail distance: Male, 0.05 mm.; female, 0.135 mm. Spicule

TABLE 2.—*Foleyella*

All measurements in millimeters	*F. americana*		*F. convoluta*		*F. duboisi*		*F. leiperi*	
	male	female	male	female	male	female	male	female
Length	23	50	15	27–32	10–13	14.9 –26.8	18.75	50
Width	0.45	0.66	0.32	0.5	0.255–0.32	0.36– 0.64	0.4	0.65
Esophagus length anterior	0.33	0.265	0.25	0.35	0.27 –0.36	0.31– 0.56	0.3	0.3
Esophagus length posterior	2.2	1.16	1.4	1.6	1.39 –1.81	1.55– 2.79	1.2	1.2
Head-nerve ring distance	0.2	0.2	0.15	0.17	0.18 –0.27	0.21– 0.37	0.25	0.25
Anus-tail distance	0.075	0.26	0.072	0.27	0.082	0.2 – 0.32	0.07	0.36
Spicule length right	0.4		0.345–0.375		0.37		0.26	
Spicule length left	0.11		0.144–0.15		0.12		0.11	
Caudal papillae, number and position	4 pr. pre-cloacal 3 pr. post-cloacal		2 pr. pre-cloacal 3 pr. post-cloacal		1 pr. pre-cloacal 2 pr. post-cloacal		2 pr. pre-cloacal 1 pr. para-cloacal 3 pr. post-cloacal	
Head-vulva distance		1.8		2		1.24–2.23		1.2
Egg sizes		(larvae) 0.018 × 0.025		(larvae) ?		(larvae) ?		(larvae) ?

TABLE 2.—*Foleyella* (*Continued*)

All measurements in millimeters	*F. ranae*		*F. scalaris*		*F. vellardi*	
	male	female	male	female	male	female
Length	8.7	16.5	34–36	35–43	36	37–50
Width	0.23	0.4	?	?	?	?
Esophagus length anterior	0.165	0.265	?	?	?	?
Esophagus length posterior	0.975	1.23	?	?	?	?
Head-nerve ring distance	0.15	0.23	?	?	?	?
Anus-tail distance	0.09–0.1	0.15	?	0.15	?	0.19
Spicule length right	0.35		0.23–0.35		0.25–0.27	
Spicule length left	0.113		0.13–0.15		0.16–0.17	
Caudal papillae, number and position	3 pr. pre-cloacal 1 pr. para-cloacal 4 pr. post-cloacal		1 pr. pre-cloacal 3 pr. post-cloacal		2 pr. pre-cloacal 2 pr. post cloacal	
Head-vulva distance		1.1				
Egg sizes		(larvae) ?		mid-esophageal (larvae) ?		post-esophageal (larvae) ?

length: right 0.088 mm.; left, 0.254 mm. Head-vulva distance, 0.625 mm. Egg sizes, 0.024 mm. by 0.035 mm.

b. *I. quadrituberculata* (Leidy, 1856) Walton, 1927 (=*Filaria solitaria* Leidy, 1856; =*F. nitida* Leidy, 1856; =*F. ranae-pipientis* Molin, 1858). From mesenteric cysts of *Amphiuma tridactylum, Rana catesbiana,* and *R. pipiens*—U. S. A. *Female:*—Length, 120 mm.; width at vulva, 1.08 mm.; length of muscular esophagus, 1.46 mm.; length of glandular esophagus, 18.3 mm.; head-nerve ring distance, 0.325 mm.; head-vulva distance, 2.7 mm.; mouth surrounded by 4 distinct papillae.

C. Position uncertain

1. *"Microfilaria"*

a. *M. tamborini* Mazza & Franke, 1927. From blood of *Leptodactylus ocellatus*—Brazil. (undescribed)

b. *M.* sp.? Mathis & Léger, 1912. From blood of *Rana limnocharis* —Tonkin, Asia. (undescribed)

B. SPIRUROIDEA

Five of the eight families of this order are represented by species found parasitic in amphibian hosts taken from all of the continents except Australia.

I. Spiruridae. Two of the four subfamilies are represented.

A. Spirurinae (*Spinitectus,* and also *Oslerus* and *Filaroides* are regarded as appendices to this subfamily.)

1. *Cystidicola*

a. *C.* sp.? (this paper). Immature male. From stomach cysts in *Eurycea bislineata*—North Carolina, U. S. A. (undescribed)

2. *Spinitectus*

a. *S. carolini* Holl, 1928. From stomach of *Triturus viridescens*—U. S. A. *Male:*—Length, 5.25–7.25 mm.; width, 0.25 mm.; pharynx length, 0.08 mm.; anterior esophagus length, 0.25 mm.; posterior esophagus length, 1.25–1.76 mm.; head-nerve ring distance, 0.13 mm.; cloaca-tail distance, 0.12 mm.; spicule lengths, 0.098 mm. and 0.17 mm.; rings of spines, 110 rows of 25–35 each; caudal papillae, 4 pairs of pre-cloacal and 5 pairs of post-cloacal; 4–5 rows of pre-cloacal tubercles. *Female:*—Length, 5.75–8 mm.; width at vulva, 0.55 mm.; pharynx length, 0.08 mm.; anterior esophagus length, 0.28 mm.; posterior esophagus length, 1.5–1.6 mm.; head-nerve ring distance, 0.16 mm.; anus-tail distance, 0.12 mm.; rings of spines, 110 rows of 25–35 each; vulva-tail distance, 0.9–1.2 mm.; egg size, 0.023 mm. by 0.036 mm.

b. *S. ranae* Morishita, 1926. From stomach of *Rana nigromaculata* —Japan. *Male:*—Length, 4–5.2 mm.; width, 0.15–0.16 mm.; pharynx length, 0.043 mm.; anterior esophagus length, 0.33–0.35 mm.; posterior esophagus length, 0.92–1.03 mm.; head-nerve ring distance, 0.086 mm.; cloaca-tail distance, 0.11 mm.; spicule lengths, 0.11–0.12 mm. and 0.9–0.93 mm.; rings of spines, 65–75 rows of 5–6 each; caudal papillae, 4 pairs pre-cloacal and 5 pairs post-cloacal; 4 rows of pre-cloacal tubercles. *Female:*—Length, 4.5–6.7 mm.; width at vulva, 0.2–0.23 mm.; pharynx length, 0.043 mm.; anterior esophagus length, 0.27–0.32 mm.; posterior esoph-

agus length, 1–1.32 mm.; head-nerve ring distance, 0.082 mm.; anus-tail distance, 0.078–0.095 mm.; rings of spines, 150–175 rows of 5–6 each; vulva-tail distance, 0.35–0.38 mm.; egg size, 0.022 mm. by 0.034 mm.

B. Hedrurinae

1. *Hedruris*

a. *H. androphora* Nitzsch, 1821. From stomach wall and spleen (?) of *Bombina bombina, Bufo calamita, Proteus angineus, Triturus alpestris, T. cristatus, T. meridionalis, T. vulgaris*—Europe.

b. *H. brevis* Walton, 1930. From stomach wall of *Triturus viridescens*—U. S. A.

c. *H. ijimai* Morishita, 1926. From duodenum wall of *Rana japonica, R. nigromaculata* and *R. rugosa*—Japan.

d. *H. siredonis* Baird, 1858. From stomach wall of *Ambystoma tigrinum*—Mexico, and *Triturus torosus*—U. S. A.

TABLE 3.—*Hedruris*

All measurements in millimeters	*H. androphora*		*H. brevis*		*H. ijimai*		*H. siredonis*	
	male	female	male	female	male	female	male	female
Length	8	10	3.8–4.5	4–5	7.5–12	11–18	8–10	16–17
Width	0.2	0.4	0.16	0.33	0.28	0.44–0.51	0.23	0.5–0.6
Esophagus length	?	?	0.95	1	1.24	1.4–1.62	0.75	1.5
Head-nerve ring distance	?	?	0.2	0.265	0.2	0.21–0.27	0.08	0.1
Head-excretory pore distance	?	?	0.265	0.335	0.4–0.56	0.51–0.67	0.075	0.08
Head-cervical papillae distance	none	none	0.16	0.185	0.43–0.47	0.53–0.56	none	none
Anus-tail distance	?	?	0.365	0.3	0.58	0.29–0.42	1	0.5
Spicule lengths	short equal		0.15		0.19–0.2		0.3	
Accessory piece length	present		0.066		0.055		0.016	
Pre-cloacal tubercles	12–15 rows		15–18 rows		12 rows		15–20 rows	
Caudal papillae, number and position	1 pr. pre-cloacal 6 pr. post-cloacal		1 pr. pre-cloacal 9 pr. post-cloacal		1 pr. para-cloacal 8 pr. post-cloacal		1 pr. pre-cloacal 10 pr. post-cloacal	
Vulva-anus distance		0.4–0.7		0.365		0.27–0.36		0.8
Caudal spike length		0.104		0.13		0.18		0.16
Egg sizes		0.046 (mammillated)		0.02 × 0.04 (non-mammillated)		0.03 × 0.04 (non-mammillated)		(mammillated)

II. Physalopteridae. Only one genus of this family is represented, North America, South America and Asia furnishing material.

1. *Physaloptera*

a. *P. amphibia* v. Linst., 1899. From esophagus of *Rana macrodon*—Luzon, Philippine Islands. *Male:*—Length, 12 mm.; width, 0.6 mm.; esophagus length, 2.8 mm.; dentition, 1 large external and 1 small tripartite internal tooth on each lip; anus-tail dis-

tance, 0.72 mm.; caudal papillae, 2 pairs lateral stalked pre-cloacals, 2 pairs lateral stalked post-cloacals, 1 pair ventral sessile pre-cloacals and 3 pairs ventral sessile post-cloacals. *Female:*—Length, 18 mm.; width at vulva, 1.05 mm.; esophagus length, 2–3 mm.; dentition, same as in male; anus-tail distance, 0.22 mm.; vulva, slightly pre-equatorial; egg size, 0.029 mm. by 0.055 mm.

b. *P. ranae* Walton, 1931. Larval forms from stomach cysts in *Rana catesbiana*—U. S. A. Length, 1.5–4 mm.; width, 0.055–0.09 mm.; muscular esophagus length, 0.07–0.12 mm.; glandular esophagus length, 0.24–0.5 mm.; head-nerve ring distance, 0.05–0.08 mm.; anus-tail distance, 0.045 mm.; dentition, 1 large external, 1 small tripartite internal, 2 minute bipartite laterals and denticular ridges on each lip.

c. *P.* sp.? Stewart, 1914. Larval forms from bladder cysts in *Bufo stomaticus*—India. (undescribed)

d. *P.* sp.? Trav., 1925. Larval forms from *Hyla faber*—Brazil. (undescribed)

e. *P.* sp.? (this paper). Larval forms from stomach cysts of *Desmognathus fuscus* and *Plethodon glutinosus*—North Carolina, U. S. A. (undescribed)

III. Camallanidae. Three of the four genera in this family are represented. South America and Australia apparently furnish hosts for members of this family.

1. *Camallanus*

a. *C. baylisi* Karve, 1930. From small intestine of *Rana tigerina*—India.

b. *C. microcephalus* (Dujardin, 1845) Railliet & Henry, 1915. From rectum of *Rana pipiens*—Wisconsin, U. S. A.

c. *C. multiruga* Walton, 1932. From intestine of "Frog"—West Africa.

d. *C. nigrescens* (v. Linst., 1906) Raill. & Henry, 1915 (= *Cucullanus nigrescens* v. Linst., 1906). From intestine of *Rana hexadactyla*—Europe.

e. *C. pipientis* n. sp. From intestine of *Rana pipiens*—Wisconsin, U. S. A.

Camallanus pipientis n. sp. (Text-fig. 2).

A large number of worms belonging to an apparently undescribed species of this genus have been collected from the rectum of *Rana pipiens* obtained in Illinois, Iowa and Wisconsin.

Male:—Length, 7.5–8 mm.; greatest width, 0.225 mm.; head capsule measures 0.11 by 0.13 mm.; number of valve ridges, 12–15; no buccal teeth; length of anterior esophagus, 0.435 mm.; length of posterior esophagus, 0.88 mm.; head-nerve ring distance, 0.2 mm.; head-excretory pore distance, 0.625 mm.; cervical papillae not found; cloaca-tail distance, 0.073 mm.; spicule lengths, right 0.55 mm. with hook 0.055 mm. from tip, and left, 0.3 mm.; stalked caudal papillae, 7 pairs precloacal, 2 pairs para-cloacal, 4 pairs post-cloacal and 1 pair subterminal.

Female:—Length, 10.25 mm.; width at vulva, 0.44 mm.; head capsule measures 0.11 by 0.145 mm.; number of valve ridges, 12–15; no buccal teeth; length of anterior esophagus, 0.457 mm.; length of posterior esophagus, 0.8 mm.; head-nerve ring distance, 0.15 mm.; head-excretory pore distance, 0.65 mm.; cervical papillae not found; anus-tail distance, 0.183 mm. (3 pronged tail); head-vulva distance, 5.5 mm.; reproduction, viviparous.

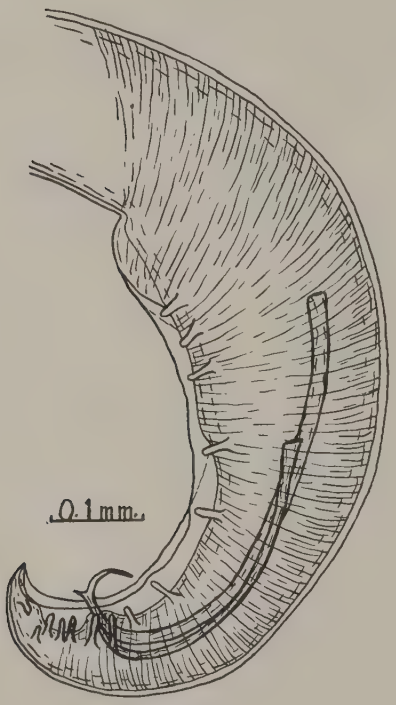


FIGURE 2

Type material in the Helminthological Collection of the U. S. National Museum.

This form differs from *C. microcephalus,* also found in *R. pipiens,* in the generally smaller size of mature specimens, and especially in the relative proportions of the lengths of anterior and posterior regions of the esophagus, the position of the excretory pore, and the number and arrangement of the caudal papillae in the male. It differs from *C. baylisi, C. nigrescens* and *C. multiruga* (the other Camallanids from amphibia) in the number of ridges in the buccal valves, in the relative proportions of the lengths of the esophageal regions, and other less important features. It also lacks the buccal teeth of *C. baylisi.*

2. *Camallanides*

a. *C. prashadi* Baylis & Daubney, 1922. From intestine of *Rana tigerina*—India. *Male:*—Length, 7.6 mm.; width, 0.2175 mm.; anterior esophagus length, 0.2625 mm.; posterior esophagus length, 0.375 mm.; head-nerve ring distance, 0.135 mm.; head-excretory

TABLE 4.—*Camallanus*

All measurements in millimeters	*C. baylisi*		*C. microcephalus*		*C. multiruga*	
	male	female	male	female	male	female
Length	8–9.5	14–20	7–10.2	10–20	9.5	8
Width	0.26–0.27	0.41	0.27	0.3–0.45	0.27	0.23
Valve ridges, number	9–11	9–11	12–14	12–14	9–11 11–9	9–11 11–9
Teeth, number	1	1	0	0	0	0
Length, Anterior Esophagus	0.48–0.51	0.63–0.65	0.36–0.5	0.42–0.5	0.4	0.43
Length, Posterior Esophagus	0.43–0.54	0.42–0.51	0.48–0.65	0.63–0.72	0.45	0.51
Head-cervical papillae distance	0.192	0.192	0.42–0.55	0.53–0.68	?	?
Head-nerve ring distance	0.139–0.195	0.23–0.247	0.22–0.28	0.36–0.4	0.5	0.75
Head-excretory pore distance	0.31	0.3675	0.25–0.3	0.4–0.42	?	?
Anus-tail distance	0.16–0.18 (bifid)	0.127–0.135 (single)	0.08 (single)	0.24–0.3 (trifid)	0.1625 (single)	0.1375 (trifid)
Caudal papillae, number and position	7 pr. precloacal 2 pr. paracloacal 6 pr. postcloacal		7 pr. precloacal 2 pr. paracloacal 3 pr. postcloacal		7 pr. precloacal 2 pr. paracloacal 7 pr. postcloacal 1 pr. sessile terminal	
Spicule lengths, Rt.	0.465		0.7 –0.84		0.335 alate	
Spicule lengths, Lft.	0.149–0.225		0.45–0.58		0.213 alate	
Head-vulva distance		5.35–6.2 viviparous		4.9–9.5 viviparous		3 viviparous

TABLE 4.—*Camallanus* (*Continued*)

All measurements in millimeters	*C. nigrescens*		*C. pipientis*	
	male	female	male	female
Length	8.2	19.7	7.5–8	10.25–10.5
Width	0.31	0.53	0.225	0.44
Valve ridges, number	9	9	12–15	12–15
Teeth, number	?	?	0	0
Length, Anterior Esophagus	0.57	0.77	0.435	0.457
Length, Posterior Esophagus	0.45	0.63	0.88	0.8
Head-cervical papillae distance	?	?	?	?
Head-nerve ring distance	?	?	0.2	0.15
Head-excretory pore distance	?	?	0.625	0.65
Anus-tail distance	0.241	0.145	0.073 (single)	0.183 (trifid)
Caudal papillae, number and position	?		7 pr. precloacal 2 pr. paracloacal 4 pr. postcloacal 1 pr. subterminal	
Spicule lengths, Rt.	0.51		0.55	
Spicule lengths, Lft.	?		0.3	
Head-vulva distance		9.75		5.5
Reproductive habit		?		viviparous

pore distance, 0.2475 mm.; head-cervical papillae distance, 0.2275 mm.; cloaca-tail distance, 0.08 mm.; spicule lengths, right, 0.24 mm. (alate), left, 0.14 mm.; accessory piece length, 0.021 mm.; caudal papillae, 7 pairs pre-cloacal, 2 pairs para-cloacal, and 5 pairs post-cloacal. *Female:*—Length, 21–25 mm.; width at vulva, 0.4–0.47 mm.; anterior esophagus length, 0.47–0.5 mm.; posterior esophagus length, 0.56–0.62 mm.; head-nerve ring distance, 0.19 mm.; head-excretory pore distance, 0.31 mm.; head-cervical pore distance, 0.35 mm.; anus-tail distance, 0.4–0.6 mm.; vulva-tail distance, 5.9–6.1 mm.; viviparous.

3. *Procamallanus*

a. *P. xenopodis* Baylis, 1930. From intestine (?) of *Xenopus müller*i—British East Africa. *Male:*—Length, 1.06–1.37 mm.; width, 0.12–0.15 mm.; length of buccal capsule, 0.08–0.1 mm.; muscular esophagus length, 0.26–0.3 mm.; glandular esophagus length, 0.2 mm.; head-nerve ring distance, 0.13–0.15 mm.; cloaca-tail distance, 0.03 mm.; spicule lengths, right, 0.1–0.11 mm.; left, 0.09–0.095 mm.; caudal papillae, 9 pairs pre-cloacal and 3 pairs post-cloacal (pedunculated) and 1 pair post-cloacal (sessile). *Female* (immature):—Length, 0.74–0.78 mm.; width at vulva, 0.09–0.1 mm.; length of buccal capsule, 0.07–0.075 mm.; muscular esophagus length, 0.22–0.24 mm.; glandular esophagus length, 0.14–0.15 mm.; head-nerve ring distance, 0.11–0.12 mm.; anus-tail distance, 0.04–0.05 mm.; vulva-tail distance, 0.31–0.41 mm.; caudal spines, 7; eggs undeveloped.

IV. Gnathostomidae. Only one genus of one subfamily is represented, the material coming from North America and Japan.

A. Spiroxinae

1. *Spiroxys*

a. *S. allegheniensis* Walton, 1930. Male only, from stomach of *Cryptobranchus alleganiensis*—U. S. A. *Male:*—Length, 24–27 mm.; width, 0.85–0.9 mm.; esophagus length, 2.35–2.4 mm.; head-nerve ring distance, 0.43–0.45 mm.; head-cervical papillae distance, 0.8–0.9 mm.; cloaca-tail distance, 0.36–0.37 mm.; spicule lengths, 1.7–2 mm.; accessory piece absent; pedunculate caudal papillae, 3 pairs pre-cloacal and 6 pairs post-cloacal; sessile caudal papillae, 1 pair pre-cloacal and 1 pair post-cloacal. *Female:*—Unknown.

b. *S. japonica* Morishita, 1926. From duodenum of *Rana japonica, R. nigromaculata* and *R. rugosa*—Japan. *Male:*—Length, 14–22 mm.; width, 0.46–0.64 mm.; esophagus length, 2.4–2.8 mm.; head-nerve ring distance, 0.85 mm.; head-excretory pore distance, 1–1.5 mm.; head-cervical papillae distance, 1.4–1.6 mm.; cloaca-tail distance, 0.33–0.37 mm.; spicule length, 2–2.2 mm.; accessory piece absent; pedunculate caudal papillae, 4 pairs pre-cloacal and 7 pairs post-cloacal. *Female:*—Length, 18–31 mm.; width at vulva, 0.71–0.98 mm.; esophagus length, 2.8–3.1 mm.; head-nerve ring distance, 0.95 mm.; head-excretory pore distance, 1.2–1.6 mm.; head-cervical papillae distance, 1.7–1.8 mm.; anus-tail distance, 0.22–0.32 mm.; vulva-tail distance, 10–11 mm.; egg size, 0.045–0.05 mm. by 0.05–0.07 mm.

V. Thelaziidae. Only one genus of one subfamily is represented by records from an amphibian host.

A. Arduenninae

1. *Physocephalus*

a. *P. sexalatus* (Molin, 1860) Diesing, 1861. An aberrant larval form from muscle cysts in body wall of "Frog"—U. S. A., Africa. Length, 1.5 mm.; width, 0.08–0.09 mm.; pharynx length, 0.025 mm.; anterior esophagus length, 0.11 mm.; posterior esophagus length, 0.2 mm.; head-nerve ring distance, 0.06 mm.; anus-tail distance, 0.045 mm.; tail with 10–15 minute terminal spines. (*vide* Walton, 1927.)

VI. Position uncertain.

1. *"Spiroptera"* s. l.

a. *S. furcata* v. Linst., 1898. Larval form from abdominal cysts in "Frog"—E. Indies. Length, 48 mm.; width, 0.87 mm.; esophagus length, 3.506 mm.; head-excretory pore distance, 0.48 mm.; anus-tail distance, 0.22 mm.; tail spike double, 0.013 mm. long.

b. *S. mugientis* v. Linst., 1898. Larval form from stomach and intestinal cysts in *Rana catesbiana*—U. S. A. Length, 52.8 mm.; width, 0.79 mm.; esophagus length, 4.061 mm.; anus-tail distance, 0.88 mm.; tail spike single, 0.16 mm. long.

c. *S. stylosa* v. Linst., 1908. Larval form from intestinal cysts in "Frog"—British East Africa. Length, 35 mm.; width, 0.66 mm.; esophagus length, 2.692 mm.; anus-tail distance, 0.321 mm.; tail rounded, spiny but not spiked.

d. *S.* sp.? (this paper). Undescribed larval forms from stomach and intestinal cysts in *Ambystoma opacum* and *Triturus viridescens*—U. S. A. (North Carolina and Mass.). Apparently not *S. mugientis* v. Linst., 1898.

C. STRONGYLOIDEA

The members of this order which are found parasitic in amphibia are almost entirely confined to the genus *Oswaldocruzia,* only one species of the genus *Amphibiophilus* apparently being recorded. Every continent except Australia is represented by examples of *Oswaldocruzia,* making this genus almost world-wide in its distribution.

I. Trichostrongylidae. This family is represented by one subfamily containing thirteen species of one genus.

A. Trichostrongylinae

1. *Oswaldocruzia*

1[1]. (*Oswaldocruzia*) sub. gen.

a. *O. collaris* Walton, 1929 (Female only). From intestine of *Rana palustris*—U. S. A.

b. *O. filiformis* (Goeze, 1782) Trav., 1917 (=*Ascaris filiformis* Goeze, 1782; =*A. tenuissima* Schrank, 1788; =*A. intestinalis* Gmel.,

1790; = *A. bufonis* Gmel., 1790 *ex. p.*; = *Cucullanus ranae* Gmel., 1790; = *Strongylus auricularis* Zed., 1800 *ex. p.*; = *Ascaris setiformis* Goeze, in Zed., 1800; = *Strongylus dispar* Duj., 1845; = *Oswaldocruzia dispar* (Duj., 1845) Trav., 1917). From intestine of *Bombina bombina, Bufo bufo, B. crucifer, B. viridis, B.* sp.?, *Hyla arborea, Pelobates fuscus, Rana esculenta, R. macrocnemis, R. temporaria, Salamandra atra, S. salamandra, Triturus cristatus* and *T. vulgaris*—Europe; from *Ceratophrys dorsata* and *Leptodactylus ocellatus*—Brazil; and from *Bufo americanus*—U. S. A. This latter record is perhaps questionable.

c. *O. insulae* Morishita, 1926. From intestine of *Bufo formosus* and *Rana rugosa*—Japan.

d. *O. leidyi* Trav., 1917 (= *Strongylus auricularis* Zed., 1800 *ex. p.*). From intestine of *Bufo americanus, Hyla cinerea* and *Pseudacris feriarum*—U. S. A.

e. *O. molgeta* Lewis, 1928. From intestine of *Triturus helveticus* and *T. vulgaris*—Wales.

f. *O. subauricularis* (Rud., 1819) Trav., 1917 (= *Strongylus auricularis* Zed., 1800 *ex. p.*; = *S. subauricularis* Rud., 1819). From *Bufo americanus, B. crucifer, B. marinus, Ceratophrys cornuta, C. dorsata, Eleutherodactylus gollmeri, Hyla faber, Leptodactylus ocellatus* and *Phyllomedusa burmeisteri*—Brazil; and from *Rana pipiens* and *Necturus maculosus*—U. S. A.

1². (*Bialata*) sub. gen.

a. *O. bialata* (Molin, 1861) Trav., 1917 (= *Strongylus auricularis* Zed., 1800 *ex. p.*; = *S. bialatus* Molin, 1861). From intestine of *Rana esculenta*—Europe; and *R. delalandii*—Africa.

b. *O. pipiens* Walton, 1929. From intestine of *Rana palustris* and *R. pipiens*—U. S. A.; and *Leptodactylus* sp.?—South America.

c. *O. natalensis* (this paper). From stomach and rectum of *Rana delalandii*—Africa.

d. *O. socialis* Morishita, 1926. From intestine of *Rana japonica* and *R. nigromaculata*—Japan.

e. *O. subventricosa* (Schn., 1866) Trav., 1917 (= *Strongylus subventricosus* Schneider, 1866). From intestine of *Bufo crucifer, Ceratophrys cornuta, Eleutherodactylus miliaris* and *Leptodactylus ocellatus*—Brazil.

f. *O. yezoensis* Morishita, 1926. From intestine of *Rana temporaria*—Japan.

g. *O.* sp.? (this paper). From intestine of "Frog"—East Africa.

h. *O.* sp.? Dikmans, 1932. From intestine of *Hyla* (sp.?)—Europe. (Deckert, 1928, reported this form as a *Pelodera* (sp.?), but Dikmans believes it to be an *Oswaldocruzia* species on the basis of the figure).

Oswaldocruzia (bialata) natalensis n. sp. (Text-fig. 3a, 3b)

The material upon which this species is based consisted of both male and female specimens taken from the stomach and rectum of *Rana delalandii* in Natal, Africa. The presence of the characteristic caudal bursa, the type of spicule, the absence of an accessory piece and also the position of the vulvar opening places this form in the genus *Oswaldocruzia,* and the presence of lateral alae relates it to the group Bialata which contains

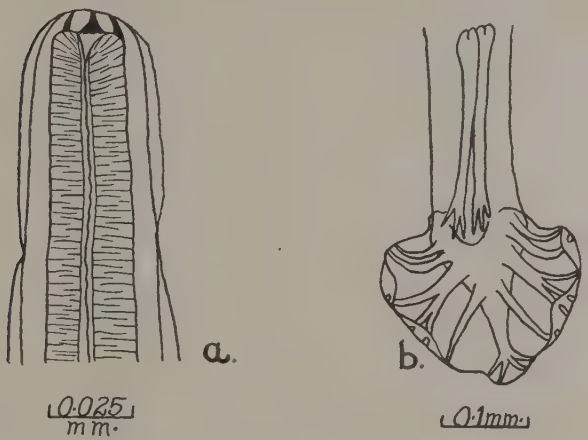


FIGURE 3a, 3b

O. bialata, O. pipiens, O. socialis and *O. yezoensis*. The size and the bursal formula in addition to the geographical distribution separates this form from all but *O. bialata*. The differences between this form and *O. bialata* are less well marked, and it may prove in the light of future study that such variations are not of specific importance. *O. bialata* is not very satisfactorily described and but little is known of it beyond the original work of Molin, 1861. Until Molin's species is more carefully studied, it seems wiser to regard this form as distinct and therefore it has been named *Oswaldocruzia* (*bialata*) *natalensis* n. sp.

The diagnostic characteristics are as follows:

Male:—Body length, 6.5 mm.; greatest width, 0.13 mm.; length of cervical inflation, 0.075 mm.; length of esophagus, 0.3 mm.; head-cervical papillae distance, 0.46 mm.; head-excretory pore distance, 0.275 mm.; head-nerve ring distance, 0.225 mm.; cloaca-tail distance, 0.225 mm.; spicules subequal, twisted, ending in three spinous processes and 0.25 mm. in length; lateral alae extend 1/3 the length of the body. Bursal formula as follows: Ventro-ventral and latero-ventral rays equal, approximate, recurved; the three laterals arising from a common trunk with the externo-lateral as the first branch; the externo-dorsal not arising from the base of the dorsal ray; the dorsal ray divided distally, each branch in turn being bifurcated.

Female:—Body length, 25 mm.; width at vulva, 0.22 mm.; length of cervical inflation, 0.1 mm.; length of esophagus, 0.42 mm.; head-excretory pore distance, 0.3 mm.; head-nerve ring distance, 0.26 mm.; head-cervical papillae distance, 0.545 mm.; anus-tail distance, 0.3 mm.; anus-vulva distance, 6 mm.; eggs, 0.0375 mm. by 0.0625 mm.; lateral alae extending 1/5 of the body length.

Type specimens are in the Collection of the British Museum (Natural History), No. 3.3.35–40.1922. From the stomach and rectum of *Rana delalandii*, Natal, S. Africa.

Oswaldocruzia (*bialata*) sp.? (Text-fig. 4a, 4b)

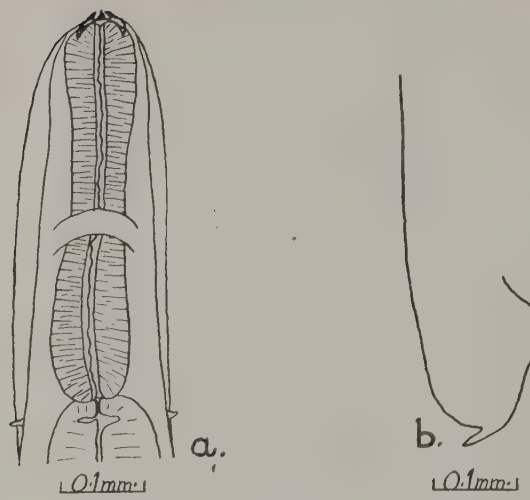


FIGURE 4a, 4b

The material consisted of female specimens taken from the intestine of a "Frog" collected near Morogoro, East Africa. These females, like those of the preceding species, resemble *O. bialata* in size and in the possession of lateral alae, but cannot be identified with any definite species in the absence of the male form.

The relative length of the lateral alae, the slightly larger cervical inflation, and the smaller size of the ova seem to be the only salient differentiations from *O. natalensis,* and further study may show these characteristics as well within the range of variation of the species named. In addition the geographical range of the host of *O. natalensis* overlaps the area from which the unidentified host of the second species was obtained, possibly a point of some significance. The measurements of these females are given as of possible aid in the eventual solution of the proper taxonomic position of the *O. bialata* and *O. natalensis* complex.

Female:—Body length, 13–27.5 mm.; width at vulva, 0.2–0.3 mm.; length of cervical inflation, 0.22–0.3 mm.; length of esophagus, 0.37–0.45 mm.; head-cervical papillae distance, 0.4–0.47 mm.; head-nerve ring distance, 0.2–0.25 mm.; anus-tail distance, 0.15–0.35 mm.; vulva-tail distance, 4.8–8.86 mm.; eggs, 0.0325 mm. by 0.055 mm.; lateral alae extending ½ of the body length.

The material described is in the Collection of the British Museum (Natural History), No. 9.8.131–133.1920. From a "Frog." Morogoro, G. E. Africa. i.iv.18 Loveridge.

II. Amidostomidae. This family is represented by only one species of one genus.

1. *Amphibiophilus*

a. *A. acanthocirratus* Skrjabin, 1916. From intestine of a member of the Ranidae—East Africa. Length: Male, 7.14–7.4 mm.; female, 11.73 mm. Greatest width: Male, 0.1 mm.; female, 0.136 mm. Esophagus length: Male, 0.425–0.45 mm.; female, 0.51 mm. Anus-tail distance in female, 0.22 mm. Vulva-tail distance, 2.3–2.9 mm. Spicule length, 0.221 mm. Accessory piece length, 0.09 mm. Egg size, 0.05 mm. by 0.085 mm.

D. DIOCTOPHYMOIDEA

The only examples of members of this order are pre-adult forms of a species usually found in fish and probably representing an accidental infestation.

TABLE 5 (a).—*Oswaldocrusia* (sub. gen. *Oswaldocrusia*)

All measurements in millimeters	*O. collaris*		*O. filiformis*		*O. insulae*	
	male	female	male	female	male	female
Length	Not known	10.75	10	11–19	8–9	12–14
Width		0.135	0.18	0.2–0.25	0.23–0.25	0.25–0.34
Esophagus length		0.4	0.6	0.6	0.45–0.5	0.34–0.5
Head inflation length		0.06	0.06	0.06	0.068–0.095	0.05–0.1
Head-cervical papillae distance		0.38	0.35	0.4–0.5	0.36–0.45	0.38–0.48
Head-excretory pore distance		0.38	0.34	0.45	0.31–0.34	0.34–0.41
Head-nerve ring distance		0.185	0.31	0.31	0.23	0.26
Anus-tail distance		0.195	0.17	0.5–0.7	0.15	0.16–0.2
Spicule length			0.23		0.21–0.22	
Vulva-tail distance		3.8		4		4.5–5.6
Lateral alae		none	none	none	none	none
Egg sizes		0.03 × 0.06		0.09 × 0.12		0.034–0.049 × 0.056–0.085

TABLE 5 (a).—*Oswaldocrusia* (sub. gen. *Oswaldocrusia*) (*Continued*)

All measurements in millimeters	*O. leidyi*		*O. molgeta*		*O. subauricularis*	
	male	female	male	female	male	female
Length	5.24	9.9	3.5–6	5–8.5	6–7	8.5–10.2
Width	0.12	0.138	0.084	0.13–0.16	0.2	0.2–0.25
Esophagus length	0.34	0.4	0.32–0.45	0.41–0.52	0.52	0.5–0.56
Head inflation length	0.02	0.07	0.025	0.03–0.04	0.07	0.07–0.127
Head-cervical papillae distance	0.285	0.3	0.23	0.3 –0.38		0.29–0.31
Head-excretory pore distance	0.25	0.28	0.22	0.3	0.3	0.29–0.31
Head-nerve ring distance	0.12	0.05	0.16–0.21	0.2 –0.25	0.263	0.26–0.28
Anus-tail distance	0.09	0.198	0.09	0.15–0.16	0.11	0.175–0.2
Spicule length	0.162		0.157–0.187		0.227–0.24	
Vulva-tail distance		3.47		1.7 –3		3.1–3.5
Lateral alae	none	none	none	none	none	none
Egg sizes		0.042 × 0.084		0.044 × 0.085		0.05 × 0.09

TABLE 5 (b).—*Oswaldocruzia* (sub. gen. *Bialata*)

All measurements in millimeters	*O. bialata*		*O. pipiens*		*O. natalensis*		*O. socialis*	
	male	female	male	female	male	female	male	female
Length	9–12	20–23	10.5	15.7	6.5	25	7–8.5	11–14
Width	.1	.1	0.132	0.15	0.13	0.22	0.11–0.18	0.27
Esophagus length	?	?	0.46	0.57	0.3	0.42	0.47–0.49	0.52–0.54
Head inflation length	?	?	0.06	0.075	0.075	0.1	0.065–0.074	0.073–0.078
Head-cervical papillae distance	?	?	0.37	0.4	0.46	0.545	0.23–0.27	0.28–0.29
Head-excretory pore distance	?	?	0.36	0.4	0.275	0.3	0.21–0.23	0.23
Head-nerve ring distance	?	?	0.25	0.225	0.225	0.26	0.165–0.17	0.19–0.2
Anus-tail distance	?	?	0.18	0.3	0.225	0.3	0.145	0.19–0.2
Spicule length	?		0.225		0.25		0.21–0.225	
Vulva-tail distance		?		6.1		6.3		4.2–5
Lateral alae	present	present	present	present	present	present	present	present
Egg sizes		?		0.035 × 0.06		0.0375 × 0.0625		0.041 × 0.082

TABLE 5 (b).—*Oswaldocruzia* (sub. gen. *Bialata*) (*Continued*)

All measurements in millimeters	*C. subventricosa*		*O. yezoensis*		*O.* sp.?	
	male	female	male	female	male	female
Length	5.8–9.5	9.2–12	6.1–6.7	9–10.4	N	13–27.5
Width	0.15	0.22	0.13	0.2	o	0.2 –0.3
Esophagus length	?	0.38–0.43	0.47	0.46	t	0.37–0.45
Head inflation length	?	0.07	0.056	0.06		0.22–0.3
Head-cervical papillae distance	?	?	0.28	0.28	K n o w n	0.4 –0.47
Head-excretory pore distance	?	0.38–0.43	0.28	0.24		?
Head-nerve ring distance	?	0.19–0.22	0.26	0.17		0.2 –0.25
Anus-tail distance	?	0.15	?	0.15		0.15–0.35
Spicule length	0.17–0.18 (no accessory piece)		0.24			
Vulva-tail distance		4.6–4.8		4.1		4.8 –8.6
Lateral alae	present	present	present	present		present
Egg sizes		0.056 × 0.085		0.04 × 0.063		0.0325 × 0.055

I. Dioctophymidae

1. *Eustrongylides*

a. *E. wenrichi* Canavan, 1929. From mesenteric, peritoneal and liver cysts of *Rana catesbiana*—U. S. A. Length: Males, 106 mm.; females, 123 mm. Width: Males, 0.86 mm.; females, 0.96 mm. Oral cavity length, 0.89 mm. in each sex. Esophagus length, 7 mm. in each sex. Head-nerve ring distance, 0.89 mm. in each sex. Two circles of head papillae in each sex. Anus terminal in both sexes and vulva subterminal. Spicule long and very thin.

E. TRICHINELLOIDEA

This order is represented by a number of species of the genus *Capillaria,* many of which are but poorly described and others are probably

only larval forms. Recent work indicated the presence of several forms in North American Amphibia and probably careful examination would reveal species of this genus elsewhere.

I. Trichinellidae

A. Trichurinae

1. *Capillaria*

a. *C. bombinatoris* (v. Linst., 1892) Trav., 1915 (=*Trichosoma bombinatoris* v. Linst., 1892). Larval forms from intestine of *Bombina bombina*—Europe.
b. *C. brevicollis* (this paper). From intestine of *Triturus viridescens*—U. S. A.
c. *C. bufonis* Morishita, 1926. From intestine of *Bufo formosus*—Japan.
d. *C. filiformis* (v. Linst., 1885). Trav., 1915 (=*Trichosoma filiforme* v. Linst., 1885). From intestine of *Triturus alpestris, T. cristatus* and *T. vulgaris*—Europe; and from *Hyla* sp.?, *Hynobius stejnegeri* and *Triturus pyrrhogaster*—Japan.
e. *C. inequalis* (this paper). From intestine of *Triturus viridescens* and *Ambystoma opacum*—U. S. A.
f. *C. tenua* Mueller, 1932. From intestine of *Triturus viridescens*—U. S .A.
g. *C. tritonis-cristati* (Dies., 1861) Trav., 1915 (=*Trichosoma tritonis* Krabbe, 1857 *ex. p.*; =*Trichosoma tritonis-cristati* Dies., 1861). Larval forms (?) from intestine of *Triturus cristatus*—Europe.
h. *C. tritonis-punctati* (Dies., 1861 after Duj., 1845) Trav., 1915 (=*Trichosoma tritonis* Krabbe, 1857 *ex. p.*; =*Trichosoma tritonis-punctati* Dies., 1861 after Duj., 1845). Female only, from *Triturus vulgaris*—France.

At least two species of Capillaria are found in American amphibia which seem to be distinct from those reported from other hosts in North America as well as from amphibia in Europe and Asia. Both belong to the sub-genus *Thomnix* on the basis of the presence of retrorse spines on the spicule sheath. In this respect they resemble many of the American and European species, including *C. tenua* from *T. viridescens* and *C. filiforme* from European and Japanese amphibia. It differs from the *C. bufonis* of Japan which lacks the spines. In size, relative position of the vulva, relative proportions of the two portions of the esophagus and number of para-esophageal cells, these two forms are not only distinctly different species but also are easily separable from the other described species. (See Table 6). These forms were obtained through the courtesy of Mr. Donald Mann, of Duke University, Durham, N. C.

Capillaria brevicollis n. sp.

Male:—Length, 4–5.5 mm.; greatest width, 0.035–0.04 mm.; head-nerve ring distance, 0.03–0.04 mm.; length of anterior esophagus, 0.219–0.327 mm.; length of posterior esophagus, 1.63–2.28 mm.; number of para-esophageal cells, all showing superficial annular constrictions, 40–45; cloaca, sub-terminal; caudal papillae, 2;

spicule length, 0.196–0.256 mm.; spicule sheath provided with retrorse spines at proximal end.

Female:—Length, 6.3–6.6 mm.; width at vulva, 0.04 mm.; head-nerve ring distance, 0.034–0.04 mm.; length of anterior esophagus, 0.49–0.52 mm.; length of posterior esophagus, 1.96–2.32 mm.; number of para-esophageal cells, 48–52, all showing several superficial annular constrictions; head-vulva distance, 2.63–2.72 mm.; anus, terminal; ova mammilated, unsegmented, and with two terminal plugs measure 0.05–0.052 mm. by 0.026 mm.

Host: *Triturus viridescens.*

Habitat: Intestine.

Locality: Woods Hole, Mass.

Collected by: D. Mann, 1931–32.

Type material deposited in the Helminthological Collection of the U. S. National Museum.

Capillaria inequalis n. sp.

Male:—Length, 6.77 mm.; greatest width, 0.045 mm.; head-nerve ring distance, 0.07 mm.; length of anterior esophagus, 0.31 mm.; length of posterior esophagus, 2.928 mm.; number of transversely annular para-esophageal cells, 52–54; cloaca, subterminal; caudal papillae, 2; spicule length, 0.238 mm.; spicule sheath provided with retrorse spines at proximal end.

Female: Length, 11.44–13.17 mm.; width at vulva, 0.054–0.064 mm.; head-nerve ring distance, 0.04–0.055 mm.; length of anterior esophagus, 0.5–0.6 mm.; length of posterior esophagus, 3.3–4.36 mm.; number of para-esophageal cells, 70–75, mostly showing superficial transverse annular rings; head-vulva distance, 4–4.52 mm.; anus, terminal; ova mammilated, unsegmented, and with two terminal plugs measure 0.055–0.06 mm. by 0.022–0.026 mm.

Hosts: *Triturus viridescens* and *Ambystoma opacum* (female only, in Ambystoma).

Habitat: Intestine.

Locality: Durham, N. C.

Collected by: D. Mann, 1931–32.

Type material deposited in the Helminthological Collection of the U. S. National Museum.

TABLE 6.—*Capillaria*

All measurements in millimeters	*C. bombinatoris*	*C. brevicollis*		*C. hepatica*		*C. filiformis*	
	larva	male	female	male	female	male	female
Length	1 [illegible]	4–5.5	6.3–6.6	8.5–9	10–12	7.86	12.48
Width	0.026	0.035–0.04	0.04	0.065	0.082	0.096	0.138
Anterior esophagus length	0.219	0.19	0.49–0.52	0.4–	0.39–	0.7	0.5
Posterior esophagus length	0.984	0.34 1.63–2.28	0.52 1.96–2.32	0.41 3.0–3.3	0.43 3.62–3.87	0.73 1.56 4.9	0.53 3.1 5.19
Para-esophageal cells, number		40–45	48–52	60–63	65–68	55–58	75–78
Head nerve ring distance		0.03–0.04	0.034–0.04	0.087 0.091	0.091 0.095	0.81	0.89
Anus position	terminal	sub terminal	termi nal	sub terminal	termi nal	sub terminal	termi nal
Spicule length		0.196–0.256		0.43–0.65		0.3	
Spicule sheath		spiny		smooth		spiny	
Caudal papillae, number		2		none		none	
Head vulva distance			2.63–2.72		1.2–1.4		0.103
Egg sizes			0.026 × 0.052		0.03 × 0.034		0.03 × 0.06
					0.065 × 0.073		

TABLE 6.—*Capillaria* (*Continued*)

All measurements in millimeters	*C. inequalis*		*C. tenua*		*C. tritonis cristati*		*C. tritonis punctati*	
	male	female	male	female	male	female	male	female
Length	6.77	11.44–13.17	7.5	10	Unknown	Not described	Unknown	16–17
Width	0.045	0.054–0.064	0.068	0.090				0.107
Anterior esophagus length	0.31	0.5–0.6	0.4	0.4				
Posterior esophagus length	2.928	3.3–4.36	3.35	3.1				
Para-esophageal cells, number	52–54	70–75	90	110				
Head-nerve ring distance	0.07	0.04–0.055	?	?				
Anus position	sub-termi-nal	termi-nal	sub-termi-nal	termi-nal				
Spicule length	0.238		0.32					
Spicule sheath	spiny		spiny					
Caudal papillae, number	2		none					
Head-vulva distance		4–4.52		3.35				
Egg sizes		0.022–0.026 × 0.055–0.06		0.024 × 0.052				0.03 × 0.065

F. APPENDIX

Under this heading are included those adult forms which are too inadequately described to be accurately placed and for which the type material has not been found, and also certain larval forms placed in the collective genus *Agamonema* since their description is too meagre to permit more accurate identification.

1. *Oxysomoides* s. l.

a. *O. terdentatum* (v. Linst., 1890) n. comb. (= *Oxysoma terdentatum* v. Linst., 1890; = *Oxysomatium terdentatum* (v. Linst., 1890) Baylis, 1927). From intestine of *Triturus cristatus*—Europe. *Male:*—Length, 15 mm.; width, 0.32 mm.; number of buccal teeth, 3; esophagus length, 1.87 mm.; no esophageal bulb; cloaca-tail distance, 0.487–0.52 mm.; caudal papillae, 3 pairs pre-cloacal, 4 pairs para-cloacal and 4 pairs post-cloacal; spicule length, 0.52 mm. *Female:*—Length, 15 mm.; width at vulva, 0.46 mm.; number of buccal teeth, 3; esophagus length, 1.875 mm.; no esophageal bulb; anus-tail distance, 0.663 mm.; vulva-tail distance, 4.616 mm.; diameter of spherical eggs, 0.098 mm.

The absence of an esophageal bulb, as pointed out by Baylis, 1927, prevents the inclusion of this form with the *Oxyurids*, as it previously had been classified. A more definite identification seems impossible at present, however, until the type material can be located and restudied.

b. *O. tuberculatus* (v. Linst., 1903) Walton, 1927. (= *Oxysoma tuberculatum* v. Linst., 1903; = *Oxysomatium tuberculatum* (v. Linst., 1903) Baylis, 1927). From intestine of *Megophrys montana*—Siam. *Male:*—Length, 3.8 mm.; width, 0.28 mm.; esophagus length, 0.95 mm.; esophageal bulb present; head-excretory pore distance, 0.4 mm.; cloaca-tail distance, 0.16 mm.; caudal papillae,

2 pairs para-cloacal and 5 pairs post-cloacal and subterminal; tail with end spike; a single pre-cloacal sucker-like structure; spicule lengths, 0.35 mm. *Female:*—(Immature). Length, 4.5 mm.; width at vulva, 0.39 mm.; esophagus length, 0.98 mm.; esophageal bulb present; head excretory pore distance, 0.42 mm.; anus-tail distance, 0.116 mm.; vulva-tail distance, 2.25 mm.; no mature eggs.

The presence of an esophageal bulb and a preanal sucker with a chitinoid border suggests a position among the Heterakidae, and the absence of caudal alae together with a spike-like tail indicates some relationship to the genus Spinicauda. In the absence of the type material it seems impossible to give a more definite identification, hence this form is left in the genus *Oxysomoides* sp. l.

2. *Agamonema*

a. *A. bombinatoris* (v. Linst., 1885) v. Linst., 1909. (=*Agamonematodum bombinatoris* v. Linst., 1885). Larvae from intestinal cysts of *Bombina bombina*—Europe. Length, 0.28 mm.; width, 0.033 mm.; anus-tail distance, 0.07 mm.; esophagus length, including posterior bulb, 0.112 mm.

b. *A. oculi-ranae* (v. Nordm., 1832) v. Linst., 1909. (=*Ascaris oculiranae* v. Nordm., 1832). Larva from eye of *Rana esculenta*—Europe. Length, 0.53 mm.

c. *A. pipae-dorsigerae* (Crep., 1844) n. comb. (=*Nematoideum pipae-dorsigerae* Crep., 1844). Larva from *Pipa pipa*—South America. (undescribed)

d. *A. proteis anguini* (Rud., 1819) n. comb. (*Dubium protei-anguini* Rud., 1819; =*Nematoideum proteis-anguini* (Rud., 1819) Dies., 1851). From *Proteus anguineus*—Europe. (undescribed)

e. *A. ranae* (Rud., 1819) n. comb. (=*Dubium ranae* Rud., 1819; =*Nematoideum ranae* (Rud., 1819) Dies., 1851). Larva from *Leptodactylus ocellatus* and *Rana* (sp.?)—Brazil. (undescribed)

f. *A. ranae-pipae* (Rud., 1810) n. comb. (=*Vermis ranae-pipae* Rud., 1810; =*Dubium ranae-pipae* (Rud., 1810) Rud., 1819; =*Nematoideum ranae-pipae* (Rud., 1810) Dies., 1851). Larva from *Pipa pipa*—Brazil. (undescribed). Probably *A. pipae-dorsigerae* and *A. ranae-pipae* refer to the same form and the names should be regarded as synonyms.

g. *A. ranae-temporariae* (Perty, 1852) n. comb. (=*Anguillula ranae-temporariae* Perty, 1852). Larva from *Rana temporaria*—Switzerland. (undescribed)

h. *A. tritonis* (v. Linst., 1877) v. Linst., 1909 (=*Agamonematodum tritonis* v. Linst., 1877). Larva from intestinal cysts in *Triturus vulgaris*—Europe. Length, 3.83 mm.; width, 0.11 mm.; esophagus length, 0.76 mm.; anus-tail length, 0.107 mm.

i. *A.* spec. 3, Hannover, 1864, v. Linst., 1909. Larvae from cysts in *Rana temporaria*—Europe. Length, 0.4–0.9 mm.

j. *A.* (sp.?) Johnston, 1914. Larva from stomach cysts of *Hyla caerulea*—Australia. Length, 3.1 mm.; width, 0.1 mm.; esophagus length, 0.43 mm.; head-nerve ring distance, 0.015 mm.; head-excretory pore distance, 0.014 mm.; anus-tail distance, 0.1 mm. This form is probably a spiropterid larva (*sensu latu*) but cannot be placed more definitely at present.

Host List

For other synonyms as recorded in earlier literature see Part I of this article, Journal of Parasitology 20: 27–29.

Rana catesbiana Shaw, 1802.
Rana delalandii (Tsch., 1838) Blgr., 1882.
Rana esculenta L., 1758.
Rana hexadactyla Lesson, 1844.
Rana (esculenta) japonica Blgr., 1882.
Rana limnocharis Boie, 1835.
Rana macrocnemis Blgr., 1885.
Rana macrodon Dum. & Bibr., 1841.
Rana nigromaculata Hallowell, 1860.
Rana palustris Le Conte, 1825.
Rana pipiens Schreber, 1782.
Rana ridibunda Pallas, 1771.
Rana rugosa Schleg., 1838.
Rana temporaria L., 1758.
Rana tigerina Daudin, 1803.
Leptodactylus ocellatus L., 1758.
Leptodactylus pentadactylus Laur., 1768.
Leptodactylus typhonius Daudin, 1803.
Eleutherodactylus gollmeri Ptrs., 1863.
Eleutherodactylus miliaris (Spix, 1824) Ptrs., 1863.
Ceratophrys cornuta (L., 1758) Wied., 1825.
Ceratophrys dorsata Wied., 1835.
Eupemphix marmoratus Fitz., 1864.
Heleioporus albopunctatus Gray, 1850.
Bufo americanus Holbrook, 1836.
Bufo bufo L., 1758.
Bufo calamita Laur., 1768.
Bufo crucifer Wied., 1821.
Bufo formosus Blgr., 1883.
Bufo marinus L., 1758.
Bufo regularis Reuss., 1833.
Bufo stomaticus Lütken, 1863.
Bufo viridis Laur., 1768.
Pseudacris feriarum (Baird, 1854) Cope, 1862.
Hyla arborea L., 1758.
Hyla caerulea White, 1790.
Hyla cinerea (Schn., 1799) Garman, 1892.
Hyla faber Wied., 1821.
Phyllomedusa burmeisteri Blgr., 1882.
Megophrys montana (Wagl., 1824) Wagl., 1824 (=*Megalophrys montana* Wagl., 1824).
Pelobates fuscus (Laur., 1768) Laur., 1768.
Bombina bombina (L., 1758) L., 1758.
Xenopus mülleri Ptrs., 1844.
Pipa pipa (L., 1758) Laur., 1768 (=*Rana pipa* L., 1758; =*Pipa americana* Laur., 1768; =*Astrodactylus pipa* (L., 1758) Wagl., 1824).
Salamandra atra Laur., 1768.
Salamandra salamandra L., 1758.
Triturus alpestris (Laur., 1768) Raf., 1815.
Triturus cristatus (Laur., 1768) Raf., 1815.
Triturus helveticus (Raz., 1789) Raf., 1815 (=*Molge palmeta* Merr., 1820).
Triturus meridionalis (Cope, 1880) Dunn, 1918.

Triturus pyrrhogaster Boie, 1826.
Triturus torosus (Baird, 1849) Stejneger & Barbour, 1917.
Triturus viridescens Raf., 1820.
Triturus vulgaris (L., 1758) Raf., 1815.
Ambystoma opacum (Gravenhorst, 1807) Baird, 1849.
Ambystoma tigrinum (Green, 1825) Baird, 1849.
Desmognathus fuscus (Raf., 1820) Baird, 1849.
Plethodon glutinosus (Green, 1818) Tschudi, 1838.
Hynobius stejnegeri Dunn, 1923.
Eurycea bislineata (Green, 1818) Dunn, 1920.
Proteus anguineus Laur., 1768 (=*Hypochthon laurentii* Merr., 1820).
Amphiuma tridactylum Cuvier, 1827.
Necturus maculosus (Raf., 1818) Raf., 1820 (=*N. maculatus* Cope, 1889).
Cryptobranchus alleganiensis (Daudin, 1803) Stejneger & Barbour, 1917.
Megalobatrachus japonicus Temminck, 1837 (=*Cryptobranchus japonicus* (Temm., 1837) Van der Hoeven, 1838; =*C. maximus* (Schleg., 1837) Chapman, 1893; =*M. maximus* (Schleg., 1837) Blgr., 1882).

Literature Cited*

1. Baird, W. 1858.
2. Baylis, H. A. 1927.
3. 1930.
4. Baylis, H. A., and R. Daubney. 1922.—Report on the Parasitic Nematodes in the Collection of the Zoological Survey of India. Mem. Ind. Mus., 8(4): 263–347, 75 figs.
5. Blanchard, R. 1905.—De una nueva especie de *Filaria* in el Sapo de Medellin. Arch. Parasitol., 9(1): 146–148. (Summary in French by Blanchard of the article by Flores, J. B. Montoya y, gives the name to the worm.)
6. Bouilliez, M. 1916.—Sur une Filaire du Crapaud de la région du Chari. Bull. Soc. Path. Exot., 9: 133–135.
7. Call, R. E. 1887.—Note on the food of *Amblystema mexicanum*. Amer. Nat., 21: 776–777.
8. Canavan, W. P. N. 1929.—Nematode parasites of Vertebrates in the Philadelphia Zoological Garden and vicinity. Parasitol., 21(1): 63–102, 5 pls.
9. Chaussat, J. B. 1850.—Note sur une nouvelle espèce de ver appartenant au genre *Trichina*, observé dans la grenouille commune (*Rana esculenta*). C. R. Soc. Biol., Paris. (1849) 1. s., 1: 84–85.
10. Creplin, F. C. H. 1844.—Endozoologische Beiträge. 4. *Amphistomum scleroporum* mihi sp. n. 5. Zweifelhafte Rundwürmer. Arch. Naturg., Berlin. 10 J., 1: 112–113, pl. 3, fig. A.
11. Deckert, K. 1928.—Mastdarmvorfall bei Laubfröschen und Nematoden. Blätt. Aquar. v. Terrarienk., 39(23): Dec. 15, 1928.
12. Diesing, K. M. 1851.
13. 1861.
14. Dikmans, G. 1932.—A trichostrongylid nematode erroneously placed in the genus Pelodera. Jour. Parasitol., 19(1): 93.
15. Dujardin, F. 1845.
16. Gedoelst, L. 1916.
17. Gmelin, J. F. 1790.
18. Goeze, J. A. E. 1782.
19. Holl, F. J. 1928.

* Citations not quoted in full in this list may be found by reference to Part I of this paper, Journal of Parasitology, 20: 29–32.

20. Johnston, T. H. 1914.—Some new Queensland Endoparasites. Proc. Roy. Soc. Queensland, 26: 76–84, 2 pls.
21. Karve, J. W. 1930.
22. Krabbe, H. 1827.—Ueber ein Trichosom in der Leber von *Triton cristatus*. Sitzungsb. d. k. Akad. d. Wissensch., Wien, math.-naturw. Cl., 25(2): 520–522.
23. Leboeuf, A., and J. Ringenbach. 1910.—Sur quelques hematozoaires du Congo (trypanosomes, microfilaires, leucocytozoaire). Ann. Inst. Pasteur, Paris, 24(12): 945–954.
24. Léger, M. 1918.—Microfilaire et filaire du Crapaud, *Bufo marinus* L. Bull. Soc. Path. Exot., 11(3): 182–187.
25. Leidy, Jos. 1852.—Some observations on Nematoidea imperfecta, and descriptions of three parasitic Infusoriae. Tr. Am. Phil. Soc., Phila., n. s., 10(2): 241–244, 10 figs.
26. 1856.
27. Leiper, R. T. 1908.—An account of some helminths contained in Dr. C. M. Wenyon's Collection from the Sudan. 3rd Rep. Welc. Res. Lab., Gordon Mem. Coll., Khartoum, pp. 187–199, 14 figs.
28. Lewis, E. A. 1928.—On a new species of the nematode genus Oswaldocruzia from the newt. Jour. Helminth., 6(2): 113–116, 3 figs.
29. Linstow, O. von. 1877.—Helminthologica. Arch. Naturg., 43 J., 1: 1–18, 26 figs.
30. 1885.—Beobachtungen an bekannten und neuen Nematoden und Trematoden. Arch. Naturg., 51 J., 1: 235–254, 3 pls.
31. 1889.
32. 1890.—Beitrag zur Kenntniss der Vogeltänien, nebst Bemerkungen über neue und bekannte Helminthen. Arch. Naturg., 56 J., 1: 171–188, 20 figs.
33. 1892.—Beobachtungen an Helminthenlarven. Arch. mikr. Anat., 39(2): 325–343, 37 figs.
34. 1898.—Nemathelminthen gesammelt von Herrn Prof. Dr. F. Dahl in Bismarck-Archipel. Arch. Naturg., 63. J., 1(3): 281–291, 21 figs.
35. 1899.—Nematoden aus der berliner zoologischen Sammlung. Mitt. zool. Samml. Mus. Naturk. Berlin, 1(2): 3–28, 78 figs.
36. 1902.—*Filaria cingula* n. sp. Zool. Anz., 25(1): 634–635, 1 fig.
37. 1903.
38. 1906.
39. 1908.—II. Helminthes. Nematoden und Acanthocephalen. Denkschr. med.-naturw. gesellsch. Jena, 13(2): 19–28, 1 pl.
40. 1909.
41. Mathis, C., and M. Léger. 1912.—Recherches de parasitologie et de pathologie humaines et animals au Tonkin. viii+451 pp., 24 figs., 14 pls. Paris.
42. Mazza, S., and I. Franke. 1927.—Microfilarias de renas (*Leptodactylus occellatus* L.) del norte argentino. Terc. Reunión Soc. Argent. Patol. Reg. Norte. Tucumán. Rev. Univ. Buenos Aires, añ. 24, 2. s., Sect. IV, 5(5): 899–901, 1 fig.
43. Molin, R. 1858.
44. 1860.—Trenta specie di nematoidi. Sitzb. Akad. Wissensch., Wien, math.-naturw. Cl., 40: 331–358.
45. 1861.—Prodromus faunae helminthologicae venetae adjectis disquisitionibus anatomicis et criticis. Denkschr. d. k. Akad. d. Wissensch., Wien. math.-naturw. Cl., 19(2): 189–338, pls. 1–15.
46. Morishita, K. 1926.—Studies on some nematode parasites of frogs and toads in Japan, with notes on their distribution and frequency. Jour. Fac. Sci. Imp. Univ. Tokyo., Sect. Zool., 1(1): 1–32, 5 pls.
47. Mueller, J. F. 1932.—*Capillaria tenua*, a new species of nematode parasitic in the newt, *Triturus viridescens*.

48. Nitzsch, C. L. 1821.—Ascaris. Allg. Encyl. Wissensch. u. Künste. Leipzig, 6: 44–49, 2 pls.
49. Nordmann, A. von. 1832.—Mikrographische Beiträge zur Naturgeschichte der wirbellosen Thiere. I. Hft., x+118 pp., 10 pls. and II. Hft., xviii+150 pp., 10 pls. Berlin.
50. Oerley, L. 1882.—Report on the nematodes in the possession of the British Museum, with a review of the classification of the order. Ann. & Mag. Nat. Hist., 5 s., 9: 301–318, figs. 1a–3b.
51. Perty, M. 1852.
52. Plimmer, H. G. 1912.—The President's Address. On certain blood parasites. Jour. Roy. Micr. Soc., London, 1912 (2): 133–150, 2 pls.
53. Polonio, A. F. 1859.
54. Railliet, A. 1916.—Sur les Filaires de Batraciens. Bull. Soc. Path. Exot., 9: 137–142, 3 figs.
55. Railliet, A., and A. Henry. 1915.—Sur les Nematodes du genre *Camallanus* Railliet et Henry, 1915. (*Cucullanus* auct., non Mueller, 1777). Bull. Soc. Path. Exot., 8: 446–452.
56. Rudolphi, C. A. 1810.—Entozoorum sive vermium intestinalium historia naturalis. II. (2), xii+386 pp. Amstelaedami.
57. 1819.
58. Schneider, A. 1866.
59. Schrank, F. von P. 1788.
60. Seurat, L. G. 1916.—Sur deux Filaires des Reptiles du Nord-Africain. C. R. Soc. Biol., 79: 1131–1136, 3 figs.
61. 1917.—Filaries des Reptiles et des Batraciens. Bull. Soc. d'Hist. Nat. de l'Afr. Nord., 9: 236–242, 8 figs.
62. Skrjabin, K. J. 1916.
63. Stewart, F. H. 1914.
64. Stossich, M. 1900.—Contributo allo studio degli eleminti. Boll. Soc. adriat. di sci. nat. in Trieste, 20: 1–9, 18 figs.
65. Travassos, L. 1915.—Contribuições para o conhecimento da fauna helmintolojica brasiliera. V. Sobre as especies brasilieras do genero *Capillaria* Zeder, 1800. Mem. Inst. Osw. Cruz, 7: 146–172, pls. 23–26.
66. 1917.—Trichostrongylidas brasilieras. Oswaldocruzia, n. gen. Braz. Med., 31(9). March, 1917.
67. 1925.
68. 1928.—Filaridés des Batraciens du Brázil. C. R. Soc. Biol., 100: 967–968.
69. Valentin, G. G. 1841.—Ueber einen Entozoon im Blute von *Salmo fario*. Arch. Anat., Physiol. u. wissensch. Med., 1841: 435–436, 1 fig.
70. Walton, A. C. 1927.
71. 1929.
72. 1930.
73. 1931.
74. 1932.—A new Nematode (*Camallanus multiruga*, sp. n.) parasitic in a West African Frog. Ann. & Mag. Nat. Hist., Ser. 10, 9: 151–154, 2 figs.
75. 1933.—The Nematodes as Parasites of Amphibia. Jour. Parasitol., 20(1) 1–32, 1 pl.
76. Yorke, W., and P. A. Maplestone. 1926.
77. Zeder, J. G. H. 1800.

Explanation of Text-Figures

Text-figure 1. *Foleyella americana*. Lateral view of tail of male.
Text-figure 2. *Camallanus pipientis*. Lateral view of tail of male.
Text-figure 3a. *Oswaldocruzia natalensis*. Anterior end of female.
Text-figure 3b. *Oswaldocruzia natalensis*. Ventral view of posterior end of male.
Text-figure 4a. *Oswaldocruzia* sp.? Anterior end of female.
Text-figure 4b. *Oswaldocruzia* sp.? Posterior end of female.

IN MEMORIAM

Theobald Smith (1859–1934)

Theobald Smith, pioneer with F. L. Kilborne in the demonstration of arthropod transmission of protozoan parasites—the piroplasms of Texas fever by ticks—died December 10, 1934, in his 76th year. Born at Albany, N. Y., July 31, 1859, and educated at Cornell University and Albany Medical College (M.D., 1883), he entered the newly organized Bureau of Animal Industry of the U. S. Department of Agriculture in 1884 to take charge of the investigations of infectious animal diseases. After eleven years of active research at Washington, he continued a long and fruitful investigational career successively at Harvard University Medical School (1896–1915) in the chair of comparative pathology, and the Department of Animal Pathology of the Rockefeller Institute for Medical Research at Princeton, N. J. (1915–1934). The latter department he organized, and while relinquishing its direction in 1929 he continued active laboratory work until a few weeks before his death. His interests—in his own words "The comparative etiology, pathology and immunology of infectious and parasitic diseases"—touched many subjects in animal parasitology. On account of his important contributions to Animal Parasitology, Dr. Smith was elected a Life Member of the American Society of Parasitologists at the Atlantic City meeting in 1932. At the time of his death he was president of the newly organized American Academy of Tropical Medicine. With the passing of Dr. Theobald Smith American science loses one of its great pioneers and leaders. In a later article in this Journal will be given an account of Dr. Smith's contribution to Animal Parasitology.

RESEARCH NOTES

THE LIFE CYCLE AND A DISCUSSION OF THE SYSTEMATICS OF THE TURTLE TREMATODE, *EUSTOMOS CHELYDRAE*

Eustomos chelydrae was first described by MacCallum (1921. Zoopath., 1: 137–284), from the small intestine of *Chelydrae serpentina.* It is evident from the figure and description that the specimen was greatly distorted. Since the characteristics and relationship of the newly created genus to other groups of trematodes were not given, it is necessary to figure and redescribe the species.

Eustomos MacCallum, 1921

Generic diagnosis.—Body elongated and covered with spines. Oral sucker larger than acetabulum, with pair of papillae at anterior lateral margin. Esophagus and prepharynx about same length. Intestinal ceca extend almost to posterior end. Genital pore preacetabular and median. Cirrus sac well developed, containing prostate gland and large, bulbous seminal vesicle, divided into anterior and posterior portions. Testes small and oblique, just posterior to end of cirrus. Ovary just posterior to acetabulum to right of median line. Vitellaria follicular. Seminal receptacle well developed. Uterus passes between testes and fills most of post-testicular region.

Type species.—Eustomos chelydrae MacCallum, 1921.

Eustomos belongs in the subfamily Plagiorchinae of the family Plagiorchidae and is closely related to the genera *Plagiorchis* Lühe (1899) and *Haplometrana* Lucker (1931). It differs from both by having oral papillae. Also, it differs from *Plagiorchis* in having a well developed seminal receptacle and from *Haplometrana* in the shortness of the esophagus and the triangular arrangement of the gonads.

Eustomos chelydrae MacCallum, 1921

Specific diagnosis.—Characters of genus: Adult (Fig. 1), 1.25–2.00 x 0.3–0.4 mm., covered with small spines. Oral sucker with two lateral papillae, larger, 0.19–0.23 mm. in diameter, than the acetabulum, 0.07–0.09 mm. in diameter. Prepharynx and esophagus about same length in relaxed specimen. Pharynx trilobed. Intestinal ceca extend almost to posterior end. Cirrus sac may lie on either side of acetabulum but usually on left, extends back of acetabulum and contains double seminal vesicle and prostate gland. Testes about same size, 0.075 mm. in diameter, oblique. Ovary about 0.07 mm. in diameter, posterior and to right of acetabulum. Seminal receptacle posterior to ovary. Vitellaria lateral, follicular, extending slightly anterior and posterior to testes. Coils of uterus pass between testes and extend almost to posterior end. Eggs operculate, 0.039 x 0.019 mm. Excretory bladder with straight stem and two lateral, tube-like arms. At posterior end the bladder opens into large muscular chamber, sometimes used like a sucker in movement. Large excretory tubes extend forward and laterally from bladder, then branch into anterior and posterior tubes. There are at least 27 pairs of flame cells.

Figure 1 represents a somewhat flattened living specimen. In fixed material the body contracts considerably, particularly in the mid-region, throwing the testes, ovary, cirrus sac, seminal receptacle and acetabulum closer together. The anterior end of the living specimen is quite extensible and motile.

The cercaria (Fig. 2), a polyadenous Xiphidiocercaria, develops in narrow, sausage-shaped sporocysts, about 0.52 x 0.09 mm. They were found in *Lymnea stagnalis appressa, Stagnicoli emarginata angulata* and *Bulmina megasoma* collected in the region of the University of Michigan Biological Station on Douglas

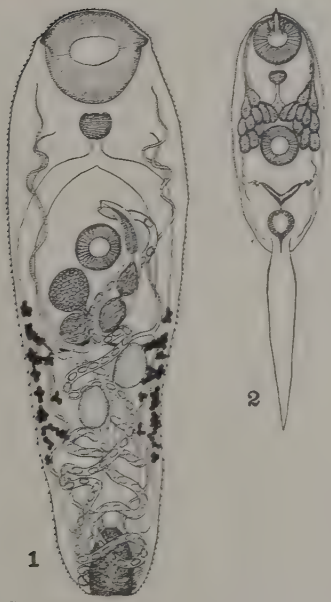


Figure 1. *Eustomos chelydrae,* adult, ventral view, × 48.
Figure 2. *Cercaria of Eustomos chelydrae,* ventral view, × 150.

Lake. The body of the cercaria, filled with small concretions and covered with small spines, measures 0.238 x 0.094 mm. The tail is without a fin fold, 0.193 x 0.030 mm. The oral sucker is larger, 0.050 mm. in diameter, than the acetabulum, 0.040 mm. in diameter. The stylet has a heavy shoulder and measures 0.028–0.030 mm. in length. There are 10 pairs of stylet glands. The intestinal ceca can not be seen in the cercaria but in the metacercaria the concretion granules collect in the bladder and the ceca can then be seen extending almost to the posterior end of the body. The excretory bladder is heavy walled and Y-shaped. The cercariae escape from the snail host in enormous numbers during the mid-morning hours. They are strong swimmers and will completely cloud the water of a half-liter container. They encyst in the respiratory basket of dragon-fly nymphs and are immediately infective. Experimentally infected nymphs were fed to 3 uninfected *Chrysemys picta.* In 10 weeks trematodes, identified as *Eustomos chelydrae,* with the uterus filled with eggs containing fully developed miracidia, were recovered from the anterior portion of the small intestine. Controls were negative. Turtles of the region, *Chrysemys* species and *Chelydrae serpentina,* were found to be naturaly infected.—Donald B. McMullen, *Department of Helminthology, Johns Hopkins School of Hygiene and Public Health.*

ODHNERIOTREMA INCOMMODUM (LEIDY, 1856), A TREMATODE FROM THE MOUTH OF *ALLIGATOR MISSISSIPIENSIS* (DAUDIN)

Canavan (Parasitol., 25: 501–509, 1933) has recently published a redescription of *Distomum incommodum* (Leidy, 1856), and proposed a new genus, *Homoscaphis,* for the species. In the writer's opinion, *D. incommodum* is congeneric

with *Odhneriotrema microcephala* (Travassos, 1922) Travassos, 1928, and the genus *Homoscaphis* Canavan, 1933, is therefore synonymous with *Odhneriotrema* Travassos, 1928.

Since Canavan has redescribed this species, a detailed description will not be given here, but attention should be called to the interpretation of certain structures, as to which there is a difference of opinion, especially since Canavan stated that the pharynx, which is well developed, was absent, mistaking it for the oral sucker. Canavan, in his description of the cirrus sac, described "a prominent vesicula seminis externus." In 4 stained specimens studied by the writer, the seminal vesicle (Fig. 1) is a coiled tube contained in the reflexed proximal end of the cirrus sac. The two ducts from the testes, the vasa efferentia, are traceable to the proximal end of the cirrus sac. No caudal process was noted on the egg (Fig. 2), nor was Laurer's canal observed by the writer, but sectioned material has not been studied. In other respects Canavan's description is in harmony with the writer's observations. Three of the 4 specimens mentioned above were collected by the writer in May and June, 1930, from the tongues of 2 alligators captured near Cape Sable and Coral Gables, Florida. The fourth specimen is from Leidy's collection (U. S. N. M., Helm. Coll. No. 85), identified by Dr. Joseph Leidy as *Distoma oricola.* Although Leidy's specimen is poorly

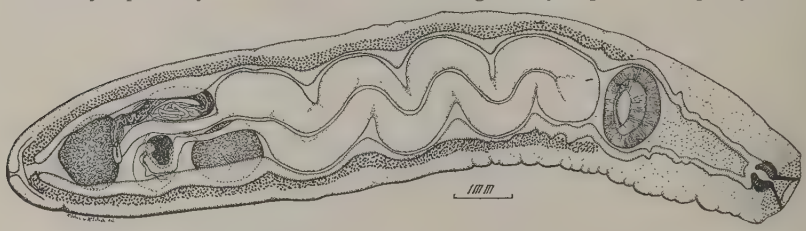


Fig. 1. *Odhneriotrema incommodum* (Leidy, 1856), ventral aspect.

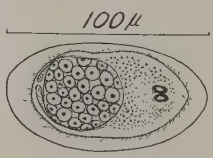


Fig. 2. *Odhneriotrema incommodum* (Leidy, 1856). Egg containing miracidium with developing sporocyst.

stained, certain structures are sufficiently clear to enable the writer to identify his material as Leidy's species, and to show that *D. incommodum* belongs to the genus *Odhneriotrema,* a genus erected by Travassos (Mem. Inst. Oswaldo Cruz, 21: 309–341, 1928) for a related species, *O. microcephala* (Travassos, 1922) from the esophagus of *Caiman sclerops.*

Odhneriotrema incommodum (Leidy, 1856) new comb. (Syns. *? Monostomum incommodum* Leidy, 1856; *Distoma oricola* Leidy, 1884; *Distomum incommodum* (Leidy, 1856) Leidy, 1891; *Clinostomum ? incommodum* (Leidy, 1856) Harrah, 1922; *Homoscaphis incommodum* (Leidy, 1856) Canavan, 1933) is closely related to *O. microcephala* (Travassos, 1922), the only other species in the genus. The two species may be separated by the position of the genital pore and the location of the ovary. In *O. incommodum* the genital pore is at or near the level of the anterior margin of the posterior testis, while in *O. microcephala* the genital pore is at the level of the anterior margin of the ovary. The ovary in *O. microcephala* is situated immediately in front of the posterior testis, but is

widely removed from the anterior testis, while in *O. incommodum* the ovary is approximately midway between the two testes, slightly nearer the anterior testis. As Canavan made no reference to the genus *Ohhneriotrema,* a brief, slightly emended diagnosis is given here.

Genus *Odhneriotrema* Travassos, 1928

Generic diagnosis.—Clinostomidae: Large flukes. Oral sucker rudimentary; acetabulum large and powerful; pharynx well developed; esophagus short or absent; intestine extending to near posterior end of body, more or less undulating in anterior portion. Genital pore ventral, median, in zone of testes. Cirrus sac well developed, reflexed, in intertesticular zone; testes tandem, in posterior third of body; ovary small, between testes; vitellaria extending from acetabulum to posterior extremity of body. Uterus forming an ascending and a descending branch of equal size; eggs embryonated, miracidium in egg showing developing sporocyst. Parasitic in mouth and esophagus of crocodylids.

Type species.—Odhneriotrema microcephala (Travassos, 1922) Travassos, 1928.—Allen McIntosh, *Zoological Division, Bureau of Animal Industry, U. S. Department of Agriculture, Washington, D. C.*

A NEW SPECIES OF TREMATODE, *UROTOCUS FUSIFORMIS* N. SP., FROM THE MOURNING WARBLER

Two specimens of a trematode belonging to the genus *Urotocus* Looss, 1899, were obtained from a bird, *Oporornis philadelphia,* found dead at the base of the Washington Monument, Washington, D. C., during the 1932 autumnal bird migration. This trematode is a new species, described here as *Urotocus fusi-*

Fig. 1. *Urotocus fusiformis* n. sp. *a.* Complete specimen, ventral aspect; *b,* Posterior extremity, dorsal aspect.

formis. The writer is indebted to Dr. Herbert Friedmann, of the United States National Museum, for the identification of the host.

Urotocus fusiformis n. sp.

Description.—Body fusiform (fig. 1), flat, spiny, 5 mm. long by 1.45 mm. wide at equator, broadest at equatorial zone and tapering towards extremities. Oral sucker terminal or slightly subterminal, 70 μ by 100 μ. Prepharynx short, 20 μ by 30 μ; pharynx 46 μ by 70 μ, surrounded by glandular mass; esophagus shorter than prepharynx, 10 μ by 30 μ; intestinal crura approximately parallel to lateral margin of body and terminating near posterior extremity, each branch filled with dark colored substance, apparently undigested blood of host. Acetabulum apparently absent, not observed in either living or fixed material. Excretory pore terminal. Testes and ovary tandem, in posterior fourth of body. Testes oval or spherical, usually wider than long; anterior testis 285 μ to 320 μ by 300 μ to 370 μ; posterior testis 212 μ to 290 μ by 300 μ to 320 μ. Cirrus pouch 250 μ by 50 μ; cirrus 150 μ long. Genital pore ventral and subterminal, about 80 μ from posterior end of body. Ovary 245 μ to 260 μ by 350 μ to 380 μ, between testes and adjacent to anterior testis. Mehlis' gland 120 μ by 270 μ, between ovary and posterior testis; Laurer's canal present. Vitellaria extracecal, extending from slightly posterior to zone of pharynx to zone of ovary. Metraterm well developed, lateral to cirrus pouch; uterus filled with eggs, ascending portion consisting of numerous coils, greatly distended with newly formed, light colored eggs, extending transversely across body, occupying all space between branches of crura and with some coils overlapping crura; descending portion of uterus ventral to ascending portion, with less extensive coils, filled with very dark brown eggs making this part of uterus conspicuous and easy to trace in its course to posterior end of body. Eggs 28 μ by 20 μ.

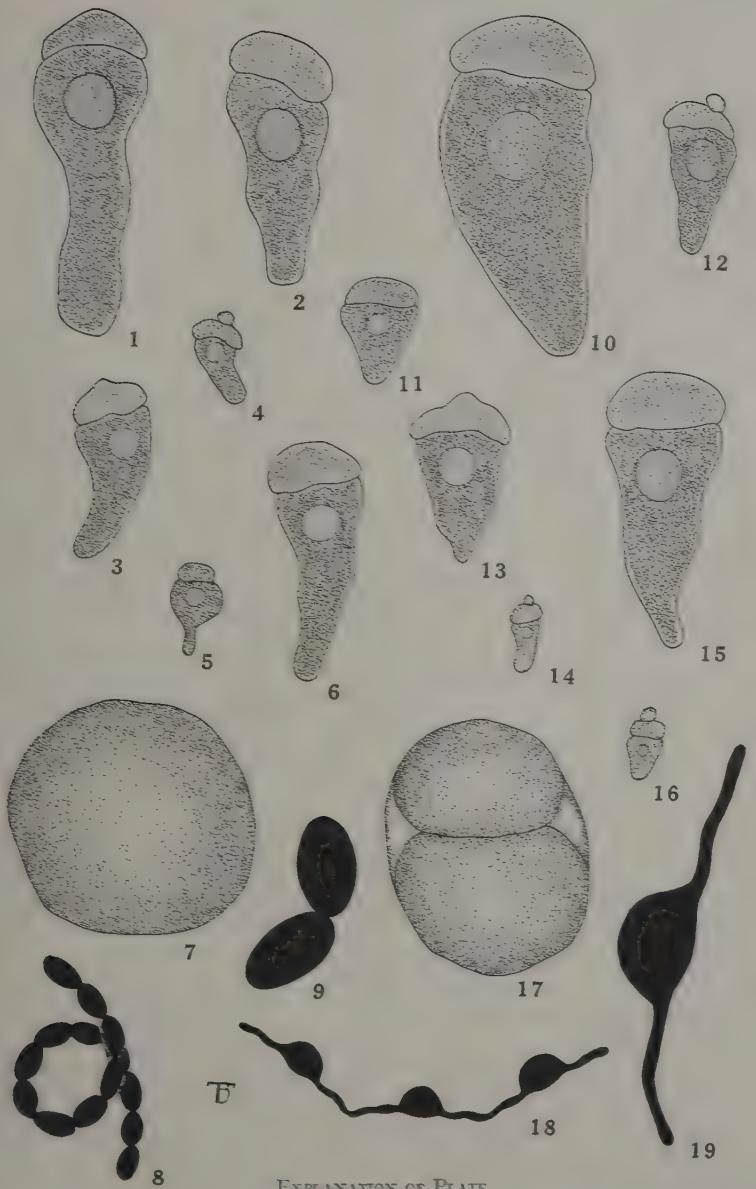
Type specimen.—U. S. N. M. Helm. Coll. No. 34309.

Habitat.—Bursa Fabricii of *Oporornis philadelphia* (Wilson), Washington, D. C.

Remarks.—*Urotocus fusiformis* is closely related to *U. rossitensis* (Muhling, 1898), the genotype and only other species of the genus. The two species may be separated as follows: *U. fusiformis* is spindle shaped, apparently has no acetabulum, and the posterior testis is removed from the tips of the crura by a distance equal to its diameter while *U. rossitensis* is oblong, has an acetabulum, and the posterior testis is between the tips of the crura. The species of the genus *Urotocus* are related to the members of the genus *Leucochloridium* in the general morphology of the reproductive system. However, the general topography of the body is very different from that of any member of that genus. In *Leucochloridium* the suckers are large and powerful, thus aiding the worm in adhering to the mucosa of the rectum. In *Urotocus* the suckers are weakly developed (the acetabulum in *U. fusiformis* is apparently absent) a fact which may be correlated with the habitat of the fluke, there being little need for strong suckers in flukes living in a sac-like structure, such as the bursa Fabricii.—Allen McIntosh, *Zoological Division, Bureau of Animal Industry, Washington, D. C.*

TWO NEW SPECIES OF GREGARINE PROTOZOA FROM THE FIREBRAT, *THERMOBIA DOMESTICA* (PACK.) (THYSANURA)

This paper presents the description of two new species of cephaline gregarines found in the domestic firebrat, *Thermobia domestica* (Pack.). The method of rearing the host is explained elsewhere (Adams, N. Y. Ent. Soc., Jour., 41: 557–562, 1933). The terms used are those of Watson (Ill. Biol. Monographs, 2, No. 3, 1916), after whom one species is named. The parasites are satisfactorily studied in salt solution kept at a concentration of one per cent.



EXPLANATION OF PLATE
From Living Specimens

Leptomastophis thermobiae sp. nov.: Figs. 1–6, trophozoites X125; 7, cyst X125; 8, spore-chain X300; 9, spores X1200.

Leptomastophis tasmaniae sp. nov.: Figs. 10–16, trophozoites X125; 17, cyst in early stage of formation X125; 18, spore-chain X300; 19, spore X1200.

These gregarines, which were reared through several generations in segregated cultures, were found to be indistinguishable in the trophozoite and cyst stages except when size and position in the host were taken into account. No genus was found to receive them. So marked, however, are the differences between their spores that two new genera are erected for them. They may, for the present, be regarded as additions to the family Gregarinidae.

Lepismatophila gen. nov.

Genotype *thermobiae*

Sporonts solitary. Epimerite a simple, symmetrical knob. Protomerite present throughout the trophozoite stage. Cyst dehiscing by rupture. Spores in uncoiling chains, ellipsoidal, devoid of processes.

Lepismatophila thermobiae sp. nov.

Cephalonts attached in ventricular caeca. Adult sporonts in main lumen of ventriculus. Deutomerite conoid, sometimes with a more narrowly conoid prolongation; protomerite sub-hemispherical. Epimerite smooth, globular, sessile. Endoplasmal mass of protomerite a pale straw-color by transmitted light. Sporonts white by reflected light. Nucleus spherical, translucent, reaching about 31 μ in diameter. Large and small trophozoites contemporaneous in host.

TABLE 1.—*Measurements in microns of six specimens (L. = length, W. = width, D. = diameter)*

Total L.	391	335	317	250	183	67
L. Protomerite	43	49	55	31	18	15
L. Deutomerite	348	286	262	219	165	52
W. Protomerite	122	153	153	104	157	30
W. Deutomerite	134	159	146	110	174	46
D. Epimerite	—	24	—	—	18	12

Fresh cysts pearly white, becoming black as spores mature; ellipsoidal, ovoidal, to sub-spherical, becoming flattened; adherent to feces or free. Hyaline layer between newly united individuals symmetrically equatorial. Average size of 20 cysts, 294 x 214 μ; largest cyst, 378 x 262 μ; smallest, 244 x 171 μ.

Spores brown, ellipsoidal, averaging 13.6 x 6.8 μ; bursting from the ruptured and recurling cyst wall in uncoiling, spiral chains.

Colepismatophila gen. nov.

Genotype *watsonae*

Sporonts solitary. Epimerite a simple, symmetrical knob. Protomerite present throughout the trophozoite stage. Cyst dehiscing by rupture. Spores in wavy chains, hat-shaped, with two curved filamentous processes at opposite ends.

Colepismatophila watsonae sp. nov.

Cephalonts in the anterior part of the post-caecal lumen of ventriculus. Protomerite sub-hemispherical. Epimerite smooth, globular, sessile. Deutomerite conoid, the posterior third varying from a broadly rounded tip to a more narrowly conoid prolongation. Nucleus spherical.

TABLE 2.—*Measurements in microns of six typical trophozoites*

Total L.	562	439	348	268	128	92
L. Protomerite	111	98	73	67	37	31
L. Deutomerite	451	341	275	201	91	61
W. Protomerite	146	134	158	171	79	55
W. Deutomerite	183	189	153	183	79	58
D. Epimerite	—	—	—	—	18	18

Comparison of table 1 with table 2 indicates that in *C. watsonae* the protomerite generally occupies a greater part of the total length than in *L. thermobiae*. Other differences are too slight to have taxonomic value.

Fresh cysts pearly white, becoming black as spores mature; ellipsoidal, ovoidal, to sub-spherical, becoming flattened; adherent to feces or free. Hyaline layer between newly united individuals symmetrically equatorial. Average size of 20 cysts, 360 x 232 μ; largest cyst, 464 x 336 μ; smallest cyst, 226 x 158 μ.

Spore brown, with a sub-hemispherical central body bearing, at opposite sides of the periphery, two curving, filamentous processes, extending in opposite directions, by which connection is kept with adjoining spores in the chain. Spore outline like profile of a broad-brimmed hat. Average dimensions of the central body, 16.5 x 9.7 μ; each process, 21.1 μ in length, 1.5 μ in thickness. Spores released in wavy chains from the ruptured and recurling cyst wall.

The writers are indebted to Dr. Elery R. Becker, under whose counsel the study was made, and to Dr. H. M. Harris for suggesting the generic names.—
J. Alfred Adams and Bernard V. Travis, *Iowa State College, Ames, Iowa.*

RAPID DEVELOPMENT OF *PSOROPHORA PYGMAEA*

In the extensive literature on mosquitoes there are many references to the rapidity with which some of the species which bred in temporary pools pass through their larval and pupal stages, but comparatively few of these give definite data on the subject. It seems worth while, therefore, to put on record a case of this sort observed near Port-au-Prince, Haiti, by Chief Pharmacist's Mate C. E. Jones, U. S. Navy, and communicated to me by Lieutenant-Commander J. E. Henry, Medical Corps, U. S. Navy. The breeding place was a small depression in flat, open pasture about 300 yards from the sea. The weather was hotter than usual and the pool was directly exposed to the sun during the whole day. This depression was dry until 9 p. m., Nov. 16, 1932. Very small larvae were observed at 9 a. m., Nov. 17. Pupae were seen in the afternoon of Nov. 18 and were brought into the laboratory the next morning. Nearly all of these pupae produced adults between 1 and 4 p. m., Nov. 19, that is, from 64 to 67 hours after the beginning of the rains which filled the depression. Lieutenant-Commander Henry sent me some of the adults from this culture, which proved to be *Psoróphora pygmaea* Theobald.—Francis M. Root, *School of Hygiene and Public Health, The Johns Hopkins University.*

PROTOZOA FROM OTHER ANIMALS IN THE CECA OF PARASITE-FREE CHICKS

In several previous reports (Hegner, 1929) the writer has described the results of inoculating intestinal protozoa from various other animals, especially birds and mammals, into parasite-free chicks, either *per os* or *per rectum*. This note adds a few records to those reports.

(1) Material from the cecum of a barred owl containing trichomonads was inoculated *per rectum* into 3 chicks; all exhibited good infections on the following day and on each of 4 examinations during the succeeding 12 days at which time the experiment was terminated. These may have been *Trichomonas oti* described by Tanabe (1926) from the screech owl.

(2) Fecal material from a chimpanzee which contained many cysts, but no trophozoites of *Chilomastix*, was inoculated into 2 starch-fed chicks; no *Chilomastix* were recovered from the chicks but trichomonads which were not observed in the inoculum, were present in large numbers on 3 examinations during the following 6 days when the experiment was terminated. Apparently the *Chilomastix* cysts did not hatch since chicks have been infected with trophozoites of *Chilomastix* from man, guinea-pig and rat (Hegner, 1929; Ball, 1931); trophozoites may encyst in the chick thus ending the infection. The trichomonads must have been very few in number in the chimpanzee to escape discovery; those

inoculated into the chick found the environment there favorable for growth and reproduction. This appears to be the first time trichomonads from the chimpanzee have been grown in chicks, although those of *Macacus rhesus* have been maintained in chicks for up to 117 days (Hegner, 1929).

(3) Ciliates and trichomonads are abundant in the stomach of ruminants. Four chicks inoculated *per rectum* with heavily infected material from cattle were found to be free from infection on 5 examinations during the succeeding 6 days.

(4) Among the intestinal protozoa of chimpanzee is the large ciliate *Troglodytella abrassarti.* Fecal material containing many of these ciliates was inoculated *per rectum* into 4 chicks. No ciliates were recovered from cecal material evacuated by the chicks about 5 hours later nor on the 4 succeeding days and none were observed at autopsy.

(5) Chicks have been inoculated with balantidial ciliates in fecal material from pigs and guinea-pigs and have retained them for periods up to 48 hours although probably no multiplication occurred. Balantidia from the pig grown in culture with an abundance of starch failed to infect 5 chicks into which they were inoculated *per rectum.*

(6) Material from the intestine of cockroaches containing *Lophomonas, Nyctotherus* and many small flagellates and amoebae did not infect 4 chicks into which they were inoculated *per rectum.* Perhaps these protozoa were unable to withstand the high temperature within the chick.—Robert Hegner, *Johns Hopkins University School of Hygiene and Public Health.*

VIABILITY OF *CHILOMASTIX MESNILI* IN WATER

It has already been demonstrated that *Trichomonas hominis* from man is quickly killed if the feces or culture medium in which it is living is highly diluted with water (Hegner, 1928). Apparently the change in osmotic pressure destroys the flagellates. The two experiments described below were carried out to determine if dilution with water would destroy trophozoites of the human intestinal flagellate *Chilomastix mesnili* also. Culture medium containing about 12 *Chilomastix* trophozoites per cmm. was diluted as follows:

Exp. 1. 0.5 cc. culture medium + 99.5 cc. tap water; shaken well and placed in an incubator at 36° C.; 3 tubes of fresh culture medium were inoculated with 1 cc. of this mixture at the end of 1, 2, 3 and 5 hours; all control tubes were positive for *Chilomastix* at the end of 72 hours; all the tubes in which the culture containing *Chilomastix* had been diluted with tap water were negative.

Exp. 2. 5 cc. culture medium + 95 cc. tap water; prepared and distributed as in Exp. 1; the control tubes were all positive and the experimental tubes all negative at the end of 72 hours.

Summary. Trophozoites of *Chilomastix mesnili* were rendered incapable of growth and multiplication in normal culture medium when the medium containing them was diluted, 0.5 cc. medium to 99.5 cc. tap water; and 5 cc. medium to 95 cc. tap water, and incubated at normal temperature (36° C.) for from 1 to 5 hours.—Robert Hegner, *Johns Hopkins University School of Hygiene and Public Health.*

INTESTINAL PROTOZOA FROM PANAMA MONKEYS

During the summers of 1931 and 1932 an opportunity was afforded through the courtesy of Dr. Herbert C. Clark to examine the digestive tract of 5 species of native monkeys at the Gorgas Memorial Laboratory in Panama. The data obtained are as follows:

(1) White-faced Monkey, *Cebus capucinus capucinus,* 12 specimens: 3 negative; 4 with *Trichomonas* only, 4 with *Giardia* only; 1 with both *Trichomonas* and *Giardia.*

(2) Brown Howler Monkey, *Alouatta palliata palliata,* 8 specimens: 2 with *Trichomonas* only; 1 with *Giardia* only; 2 with *Trichomonas* and *Giardia*; 1 with *Trichomonas* and an undetermined species of *Endamoeba*; 1 with *Trichomonas, Giardia, Chilomastix* and *Retortamonas.*

(3) Black Spider Monkey, *Ateles dariensis,* 4 specimens: 1 negative; 1 with *Trichomonas* only; 1 with *Trichomonas, Giardia* and *Balantidium;* 1 with *Trichomonas, Balantidium* and *Taliaferria.*

(4) Marmoset, *Leontocebus geoffroyi,* 5 specimens: 2 negative; 3 with *Trichomonas.*

(5) Night Monkey, *Aotus zonalis,* 2 specimens: 1 negative; 1 with *Trichomonas* only.

Summary. Five species and 31 individual monkeys contained 7 genera of intestinal protozoa. The number of species and individuals infected with each genus of protozoa is as follows: *Trichomonas,* 5 species and 18 individuals; *Giardia,* 3 species and 10 individuals; *Balantidium.* 1 species and 2 individuals; *Taliaferria, Chilomastix, Endamoeba,* and *Retortamonas,* 1 species and 1 individual each.—Robert Hegner, *Johns Hopkins University, School of Hygiene and Public Health.*

CAPILLARIA INFESTATIONS IN NEW JERSEY PHEASANTS

A "diphtheritic" disease was observed in seven young adult ring-necked pheasants (*Phasianus colchicus torquatus*) which were procured from the State Game Farm at Forked River, N. J., in November, 1925. Nematodes associated with the lesions in the upper digestive tract have been identified as *Capillaria annulata* (Molin, 1858) syn. *Trichosomum strumosum* Reibisch, 1893, and *Trichosoma delicatissimum* Perroncito and Tomiolo, 1899. In making this diagnosis, reliance has been placed on the cervical dilatation of the cuticula as a specific character (Cram: North Amer. Vet. 7: 46–48, 1926), as well as in the general features of size, shape and location of the parasite.

A fibrinous pseudomembrane was present, covering the mucosa of the buccal cavity and tongue, and to a lesser extent the esophagus, proventriculus and crop. The parasites were partially embedded in the pseudomembrane and projected discontinuously into the mucosa. They were present in moderate numbers in the crop of one pheasant. Microscopic examination revealed extensive necrosis of the mucosa, with marked desquamation of the epithelium in all sections, being practically complete in some from the esophagus. The galleries formed by the worms in the mucosa did not appear to be necrotic. The tissues were hyperemic and there was a diffuse infiltration of eosinophilic leucocytes with a more pronounced, though irregular, infiltration of plasma cells in the musoca and underlying submucosa. Essentially the picture agreed with that described by Hung (North Amer. Vet., 7: 49–50, 1926) for *C. annulata* infestations in turkeys although no caseous degeneration of the lymph follicles was observed in the present cases and the cellular infiltration was with plasma type cells rather than with lymphocytes.

Galli-Valerio (Il Moderno Zooiatro, 8: 22–23, 1897) reported that *Trichosoma longicolle* Rud., 1819 [=*Capillaria longicollis* (Rud., 1819)] produced marked morbidity in pheasants, with lesions in the ingluvies accompanied by round cell infiltration. He made no mention of a necrotic exudate. This seems to be the only recorded case in which *C. longicollis* has been reputed to be a parasite of the upper digestive tract. From the morphological data presented, it is not possible to allocate the species with certainty but it seems probable that either *C. annulata* or *C. contorta* (Creplin, 1839) was the parasite involved. Both have been reported from the anterior digestive tract of pheasants.

It may be that Galli-Valerio recognized the parasite as the one described by Molin in 1858, but did not agree with the view of Molin (Denkschr. d. k. Akad. d. Wissensch., math.-naturw. Cl., v. 19, 2. Abt., pp. 189–338, 1861) that *T. longicolle* Rud., 1819, was a synonym of *T. annulatum,* accepting instead the identity

of the two forms and choosing *T. longicolle* as the name indicated by priority without assuring himself of the identity of the two parasites. They are now recognized as distinct species.

Capillaria annulata has not been previously reported from pheasants in this country. As it is known to be a parasite of pheasants and chickens in Europe and elsewhere, and has been reported from chickens, turkeys, quail, grouse and partridge in the United States, it is clear that the nematode has a wide geographical distribution. It is probable that ingestion of infective eggs will produce infestations in many gallinaceous birds.

Besides *C. annulata,* the capillarid species, *C. contorta, C. longicollis* and *C. meleagris-gallopavo* have been reported as pheasant parasites, the latter two being from the small intestine.

I am indebted to Dr. Theobald Smith for the pheasant material described in this communication.—George L. Graham. *The Rockefeller Institute for Medical Research, Princeton, N. J.*

REVIEWS

Handbook of Human Parasitology. Volumes 1 and 2. By Sadamu Yokogawa and Kaoru Morishita, Publisher: Tohodo, Tokyo, Japan.

This handbook of human parasitology written in Japanese is planned to include all phases of the subject. Volumes 1 and 2 published in 1931 and 1933, respectively, cover the field of human helminthology. Volume 1 consists of 527 pages, 119 of which are given over to bibliography, and has 191 illustrations. Volume 2 has 603 pages, 137 of which are bibliography, and 200 illustrations. Volume 1 includes the introduction to human parasitology, a special introduction to human helminthology, and the sections on trematodes and cestodes of man. Volume 2 takes up the nematodes and leeches and has chapters on intermediate and reservoir hosts of human helminths and on diagnosis and technic. Volumes 3 and 4 on protozoology and medical entomology are in process of preparation.

The two volumes already published cover systematically both the medical and zoological phases of human helminthology. The different species are taken up from the view point of morphology, life history and distribution, and the pathology, diagnosis, treatment, and control of the diseases which they produce are fully discussed. While the discussions are primarily based on the very extensive recent literature of Japan the authors show a good acquaintance with the researches published in other languages. For example the references on *Schistosoma japonicum,* which cover eight pages of text, include in addition to references in Japanese almost 40 in other languages. Also in the 38 pages of references on hookworm and hookworm diseases found in volume 2 are found listed almost all the important recent publications on this subject.

The bibliography forms a very important part of the two volumes covering in all 256 papges. It is arranged according to subjects and will serve not only to place the foreign literature before the Japanese students, for whom of course the book is primarily intended, but will also serve as a perfect gold mine of information on the very extensive Japanese literature in human helminthology. The illustrations are on the whole excellent and well selected. Many of them are original drawings and photographs from material not readily accessible outside the Orient.

The publication of this handbook is further evidence of the important part that Japanese workers are having in the development of human helminthology and will be a great aid to workers in other countries in getting acquainted with the Japanese researches.—W. W. Cort.

Plant Parasitic Nematodes and the Diseases They Cause. By T. Goodey, D.Sc. E. P. Dutton and Co., New York. 1933. xx and 306 pp., 136 figs. Price $6.75.

For the first time the science of plant parasitic nematodes and the diseases they cause is treated in book form, a highly commendable undertaking for which the author and publisher must be congratulated. Anyone who knows how scattered the material on this subject is throughout scientific literature, will recognize the completeness with which it has been assembled, and anyone acquainted with the often fragmentary and preliminary character of this literature will compliment the author on his presentation of the subject. The book undoubtedly fills an urgent need. It is well bound, printed on good paper, generously provided with figures and will greatly help to promote work in this field. This reviewer sees an early need for a new edition. It is mainly for this reason that suggestions for improvement are presented in the following critical remarks.

Probably cognizant of the fact that this book will be used widely by plant pathologists and other investigators often not very familiar with the subject matter, the author has arranged the contents in a rather unusual way, *i.e.,* the

division into chapters is not based on a uniform principle. One chapter is based on disease symptoms produced on the hosts, others are based on parts of plants affected (roots, shoot structures), and still others on taxonomic and ecological relations of the parasites. This arrangement is not favorable for a uniform and concise presentation of the subject matter.

Chapter I.—"General Considerations" outlines the structure of a nematode, the technique of the study of plant parasitic forms and their taxonomic standing. The paragraph on the general structure of a nematode seems to this reviewer to be the least satisfactory in the entire book. Statements such as: "These muscle cells are found underlying the *dorsal* and *ventral fields* (italics by the reviewer!) of the body but not under the lateral fields" (page 3—top), or "Head papillae are often present and are commonly six in number" (page 3—line 8), or, speaking of the buccal cavity, "Part of it may be surrounded by the oesophageal muscles, when it is called a pharynx" page 4—top) are not quite in agreement with reality and an up-to-date morphological conception of a nematode. There is confusion as to what is called a bursa. On page 5 this statement occurs: "The cuticle of the male tail may be raised into a lateral wing or ala on each side of the body. These wings may completely surround the tip of the tail and form a bursa." Commonly this structure is termed bursa even if not surrounding the tip of the tail; but the author himself does not always stick to this unusual definition, for example, on page 15, Fig. 5-F, he calls a bursa what, according to his definition, should be called merely wings or alae. Figure 1 is apparently a simplified copy of a diagrammatic figure originally published by Cobb. In such figures the association of characters that are never found together on an actual specimen, like caudal glands and bursa as shown in the schematic tail end of a male in this figure, does not seem good policy. In addition, this same figure is unfinished as to the cloacal and anal connections of the intestine as well as the ejaculatory duct.

The paragraph on measurements and proportions in the same chapter proposes, on page 10, a number of new measure relations added to those which are known as the de Manian formula (or α, β, γ system). There is δ = total length of the body/distance of vulva from tip of tail. No reason can be seen why such a relation should be exposed. The position of the vulva is commonly, and even in the present book, and to better advantage expressed in per cent of the total length and used so in connection with the aforementioned de Manian formula and with Cobb's percentage formula. Why then is this relation (δ) suggested, and not even used in the present book? The same is true for the proposed relations ε = total length of the body/distance anterior end to excretory pore, and ζ = length of oesophagus/length of buccal stylet. Cobb's conventional-signs formula on page 11 had better be discarded from a book of this type because it will be Sanscrit to most users. It is regrettable that various types of measurement formulae are used instead of a single system; they tend to add to the compilatory character of this book. The author apparently wished to give the measurements as they were originally expressed by the investigator, but by doing so, the difficulties for the reader of the book are much increased.

Chapters II–VIII cover the various plant parasitic nematodes, including what the author calls "not obligate parasites." The division of these chapters is illogical in part. Chapter II headed "Species of *Anguillulina* causing galls on Shoot Structures" does not include all such forms since species of *Anguillulina* producing galls on shoot structures are also treated in Chapter III, for example, *Anguillulina dispaci* which on many plants produces galls (plantain, strawberries, narcissus, ranunculus, etc.) and *Anguillulina darbouxi* and *Anguillulina fucicola*, etc. Chapter III has the heading, "Species of *Anguillulina* parasitic in Shoot Structures." Certainly species causing galls on shoot structures (Chapter II) are also parasitic. Then follow Chapter IV ("Root Parasites: Species of *Anguillulina* and *Tylenchulus semipenetrans*, the Citrus Root Nematode"), Chapter V ("Root Parasites: Species of *Heterodera*"), Chapter VI ("Root Parasites:

Species of *Heterodera*" (concluded)), Chapter VII ("Species of *Aphelenchoides*") and Chapter VIII ("Nematodes other than Obligate Parasites"). This arrangement detrimentally separates matters which otherwise belong together without serving the purpose which probably instigated the arrangement. A final chapter—Chapter IX—is devoted to "Biologic Races in Plant Parasitic Nematodes." Each chapter concludes with a bibliography. This of course involves certain repetitions, but may assist plant pathologists in finding the most important literature references adjacent to the forms which they are studying.

The author shows himself well acquainted with the literature. Often, however, he quotes almost too much, and in many instances personal experience seems to be lacking. Authors are often cited without the necessary criticism, for example, the recommendations quoted as to the use of formaldehyde for the control of the root knot nematode, on page 174, are practically useless since the solutions recommended are too weak to be of any help. Temperatures are given sometimes in Fahrenheit, sometimes in Centigrade.

Host lists would be more practical if arranged alphabetically according to genera and species rather than according to families.

The regular text has few misprints (*e.g.*, p. 214 quaranting instead of quarantining). Misprints are more numerous in the bibliography, especially the literature references in French and German, *e.g.*, on pages 46, 97, 157, 224 and 279. The abbreviations used for periodicals are often unusual, making deciphering difficult. The figures are in the main excellent. However, figures 25, 62, 105, 114 and some others are badly redrawn copies. Here and there the unfinished character of a figure must be criticized, for example, on page 268, Fig. 126, D—neither intestine nor ejaculatory duct is connected with the anus; the same is true of Fig. 127, D, Fig. 119, etc. This would be misleading to a beginner or a person not acquainted with nematodes.

Anguillulina multicincta (Fig. 96, page 231) is *Anguillulina dihystera* (Cobb). *Anguillulina multicincta* (Cobb) is a synonym of *Anguillulina robusta* (de Man, 1876). The ascription of a distinct postbulbar region of the oesophagus to *Aphelenchus* (second sentence of the paragraph on *Paraphelenchus,* page 255) is incorrect. On page 83, line 11 from the bottom, but also elsewhere, three crescentic thickenings of the lumen of the median oesophageal bulb are spoken of. It is the wall of the oesophageal canal that has crescentic thickenings. On pages 30 and 36 Fine Bent-grass is given as *Agrostis vulgaris* With. (*A. tenuis* Sibth.); on page 40 as *Agrostis alba* L. (*A. vulgaris* With.); but on page 30, lines 5 and 6 it appears apparently as two species, again as *Agrostis alba* and *Agrostis vulgaris*. *Agrostis stolonifera* (page 26) becomes *Agrostis alba* (*stolonifera*) (on page 28) and again it is apparently referred to as *Agrostis polymorpha* (*stolonifera*) (on page 30). Nomenclatorial discussions, as given on page 13, could be omitted to advantage in another edition.

The reviewer is sorry that Staniland's latest results on the treatment of narcissus bulbs with hot water are mentioned in a special addendum to the book when they are so contrary to all previous experience, and have been since disproved in their general application. The 3-hour hot-water-treatment of narcissus bulbs at 110–111½° F. is known to be often unsuccessful in the control of *Anguillulina dipsaci*. Staniland's recommendations of a much shorter period of treatment are misleading because they are based on observations of active specimens of the nematode only. The failures of the much longer treatment as it is generally used heretofore, are today known to be due to the presence of so-called dormant and therefore more resistant specimens. Staniland ignores the presence of these in the bulbs and ignores the fact that these specimens will be found mainly in the neck and surface regions of the bulb and not in the center. If proper steps are taken to revive these dormant specimens, for example, by a previous submersion of the bulbs in water at 70–80° F. for 2 or 2½ hours, then the time of the hot-water treatment may be considerably shortened, but Staniland

apparently was unaware of the complexity of this problem. The quotation of his results in the present book may do more harm than good.

This criticism does not intend to impair the value of the present treatise. Being the first and only one covering the entire subject of plant parasitic nematodes, it is indispensable for anyone working in this and related fields.—G. STEINER.

JOURNAL OF PARASITOLOGY

ANNOUNCEMENT OF CHANGE IN SUBSCRIPTION PRICE AND TIME OF PUBLICATION

The changes in policy of the JOURNAL OF PARASITOLOGY already announced go into effect with the present number. The JOURNAL is to appear bimonthly and the volume will coincide with the calendar year. Under this arrangement volume 21 will be published in six numbers, appearing in February, April, June, August, October, and December. It is also planned to increase the number of pages per volume.

These changes make necessary an increase in subscription price. Beginning with volume 21 the subscription price of the JOURNAL OF PARASITOLOGY has been raised to four dollars ($4.00) (Foreign, $4.25) per volume.

W. W. CORT, *Chairman,*
Editorial Committee

INTE

0022-3395

apparently was unaware of the complexity of this problem. The quotation of his results in the present book may do more harm than good.

This criticism does not intend to impair the value of the present treatise. Being the first and only one covering the entire subject of plant parasitic nematodes, it is indispensable for anyone working in this and related fields.—G. STEINER.

JOURNAL OF PARASITOLOGY

ANNOUNCEMENT OF CHANGE IN SUBSCRIPTION PRICE AND TIME OF PUBLICATION

The changes in policy of the JOURNAL OF PARASITOLOGY already announced go into effect with the present number. The JOURNAL is to appear bimonthly and the volume will coincide with the calendar year. Under this arrangement volume 21 will be published in six numbers, appearing in February, April, June, August, October, and December. It is also planned to increase the number of pages per volume.

These changes make necessary an increase in subscription price. Beginning with volume 21 the subscription price of the JOURNAL OF PARASITOLOGY has been raised to four dollars ($4.00) (Foreign, $4.25) per volume.

W. W. CORT, *Chairman,*
Editorial Committee